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Air Pollution and Acid Rain
Report No. 16

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COMPARATIVE ANALYSES
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UNITED STATES
DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

EASTERN ENERGY AND LAND USE TEAM
Route 3, Box 44
Kearneysville, West Virginia 25430

Dear Colleague:

The Eastern Energy and Land Use Team (EELUT) is pleased to provide you this report on the comparative analyses of fish populations in naturally acidic and circumneutral lakes in northern Wisconsin. This report is the sixteenth in our series dealing with air pollution and acid rain. Other reports previously issued are listed on the inside front cover.

In this research fish populations in six naturally acidic (autumnal pH 5.6-6.0) and six circumneutral (autumnal pH 6.7-7.5) clear-water lakes in north central Wisconsin were studied, and characteristics of fish populations and communities in the lakes were compared. Differences in 1) species composition and richness of fish communities, 2) growth and condition of bluegills, 3) serum calcium concentrations of bluegills and white suckers prior to spawning, 4) accumulation of potentially toxic metals by bluegills and walleyes, and 5) bilateral asymmetry of anatomical characters of bluegills and white suckers between the two groups of lakes were statistically compared and evaluated in terms of pH-related effects.

Your comments and suggestions on this report are welcomed.

Sincerely,

R. Kent Schreiber

R. Kent Schreiber
Acting Team Leader, EELUT

FWS/OBS-80/40.16
September 1983

Air Pollution and Acid Rain
Report No. 16

COMPARATIVE ANALYSES OF FISH POPULATIONS IN NATURALLY ACIDIC AND CIRCUMNEUTRAL LAKES IN NORTHERN WISCONSIN

by

James G. Wiener

U.S. Fish and Wildlife Service
Columbia National Fisheries Research Laboratory
Field Research Station
P.O. Box 936
La Crosse, Wisconsin 54601

Project Officers

Paul J. Rago/R. Kent Schreiber
Eastern Energy and Land Use Team

Performed for:
Eastern Energy and Land Use Team
Division of Biological Services
Research and Development
Fish and Wildlife Service
U.S. Department of the Interior
Washington, DC 20240

Fish and Wildlife Service

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DISCLAIMER

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EXECUTIVE SUMMARY

Recent investigations of surface water and precipitation chemistry have aroused concern about the potential effects of acidic precipitation on fishery resources in the north central United States. Hundreds of lakes in north central Wisconsin are poorly buffered, naturally acidic, and susceptible to acidification. Precipitation in this area is acidic, with volume-weighted, annual mean pH ranging from about 4.5 to 4.7 and annual wet deposition of sulfate ranging from about 250 to 380 eq/ha.

The primary objective of this study was to determine whether fishes inhabiting naturally acidic lakes in north central Wisconsin exhibit symptoms of acid/metal stress. If acid/metal stress is evident, acidification of these lakes would probably have immediate adverse impacts on fish populations. In 1980, comparative analyses of fish populations were initiated in six naturally acidic (pH range, 5.1-6.0) and six circumneutral (pH range, 6.7-7.5) clear-water lakes in north central Wisconsin during 1980. The two groups of lakes (here termed acidic and circumneutral) were morphologically similar and contained lakes of the same hydrologic types, but differed substantially in pH, alkalinity, calcium concentration, and related chemical characteristics. Mean waterborne calcium concentrations in near surface (0.5 m) samples during summer ranged from 1.0 to 1.7 mg/l in the naturally acidic lakes and from 4.2 to 10.1 mg/l in the circumneutral lakes. Dystrophic (bog) and winterkill lakes and lakes located on recently disturbed watersheds were excluded from study. Selected characteristics of fish populations and communities in the naturally acidic and circumneutral lakes were quantified. Differences in (1) species composition and richness of fish communities, (2) growth and condition of bluegills, (3) serum calcium concentrations of bluegills and white suckers prior to spawning, (4) accumulation of potentially toxic metals by bluegills and walleyes, and (5) bilateral asymmetry of anatomical characters of bluegills and white suckers between the two groups of lakes were statistically compared and evaluated in terms of pH-related effects. Because many water chemistry parameters (e.g., alkalinity, metal concentrations and forms, and calcium) vary concomitantly with pH, it is emphasized that the naturally acidic and circumneutral lakes differed mainly in pH-related chemical characteristics and not solely in pH. This project was conducted with cooperation from personnel of the Wisconsin Department of Natural Resources, North Central District and the U.S. Environmental Protection Agency, Environmental Research Laboratory in Duluth, Minnesota, who are studying surface water and precipitation chemistry in this area.

Comprehensive surveys of fish communities revealed that water chemistry factors, believed to be pH and waterborne calcium concentration, strongly influenced the species composition and richness of fish communities in these lakes. Fish communities in the naturally acidic lakes contained significantly fewer species than those in the circumneutral lakes. Common as well as rare species occurred with lower frequency in the acidic lakes than in the circumneutral lakes. Certain taxa, such as minnows (Cyprinidae) and darters (*Etheostoma* spp.), were well represented in the circumneutral lakes but were either absent or rare in the naturally acidic lakes, probably because of pH-related stress in conjunction with low waterborne calcium concentrations. Species richness of fishes other than minnows and darters was also significantly lower in the acidic than in the circumneutral lakes. The differences in species composition and richness of fish communities between the two groups of lakes did not appear to be related to differences in physical habitat characteristics, past fish migrations, or productivity.

Growth and condition of bluegills (*Lepomis macrochirus*), which occurred in five acidic and six circumneutral lakes, were compared. Although mean condition factors and mean back-calculated total lengths at ages 1 to 4 varied significantly among lakes, the differences were not related to lake pH. Rather, the ranks of mean condition factors and back-calculated lengths at ages 2, 3, and 4 were negatively correlated with relative density of bluegill populations among the lakes. Because of the dominating effect of density, growth rates and condition factors are not useful as indicators of chronic, pH-related stress on bluegill populations.

Serum samples were collected from mature white suckers (*Catostomus commersoni*) and bluegills from selected Wisconsin lakes during spring 1981, prior to spawning. Female white suckers were collected from a lake with neutral pH (7.0) and from two naturally acidic lakes (pH 5.6). The mean serum calcium concentration in female white suckers from the neutral lake was significantly greater than in those from the acidic lakes, whereas mean concentrations in female white suckers from the two acidic lakes did not differ. Serum calcium concentrations in female bluegills from four acidic and four circumneutral lakes varied among lakes; however, no relation between serum calcium concentration and either lake pH or waterborne calcium concentration was apparent. In contrast, a strong negative rank correlation between mean serum calcium concentration and relative density of bluegills in the eight lakes was observed, suggesting that the timing of the reproductive cycle of bluegills is related to nutritional status. Mean serum calcium concentrations in mature male bluegills, measured in samples from one acidic and one circumneutral lake, were substantially less than in female bluegills from the two lakes, which is normal prior to spawning. It was hypothesized that white suckers, which are presumably more acid-sensitive than bluegills, are experiencing chronic pH-related stress in naturally acidic Wisconsin lakes with annual pH ranges of 5.6-5.8 or less and with waterborne calcium concentrations less than 2.0 mg/l. In an acidified Ontario lake studied by Canadian investigators, mature female white suckers exhibited depressed serum calcium concentrations, which were associated with failure to produce viable eggs. The utility of serum calcium concentrations, measured prior to spawning, as a measure of pH-related stress on populations of white suckers and other fishes with synchronous reproductive cycles merits further study. However, this measure is probably not useful for

evaluating chronic acid stress on fish species, such as the bluegill, which have asynchronous reproductive cycles.

Dual composite samples of whole bluegills from five acidic and five circumneutral lakes were analyzed for aluminum, cadmium, copper, manganese, lead, vanadium, and zinc. All bluegill samples analyzed were composed of four-year-old fish, with the exception of the samples from one acidic lake, which contained six- and seven-year-old fish. Concentrations of cadmium, manganese, lead, vanadium, and zinc in bluegills varied significantly among lakes, whereas concentrations of aluminum and copper did not. Mean concentrations of cadmium and lead in bluegills from the ten lakes were inversely (rank) correlated with both pH and waterborne calcium concentration. Mean concentrations of manganese, vanadium, and zinc in bluegills were unrelated to lake pH and did not exhibit the many-fold (~20X) variation among lakes shown by cadmium and lead. Internal concentrations of manganese, vanadium, zinc, and other essential trace elements in fishes appear to be homeostatically controlled and are consequently less variable than those of the nonessential elements cadmium and lead. The mean and maximum concentrations of cadmium and lead in whole bluegills from the naturally acidic lakes were similar to, or exceeded, values reported for whole bluegills from U.S. lakes and rivers contaminated by metal-containing industrial effluents and urban runoff. In contrast, cadmium and lead concentrations in bluegills from the circumneutral Wisconsin lakes were similar to values reported for bluegills from uncontaminated waters.

Mercury concentrations were measured in axial (edible) muscle tissue of four-, five-, and seven-year-old walleyes (*Stizostedion vitreum vitreum*) from two naturally acidic and three circumneutral lakes. Mercury concentrations in walleyes from the two acidic lakes (range, 0.41-1.74 µg/g wet weight) were substantially higher than in walleyes from the three circumneutral lakes (range, 0.22-0.57 µg/g wet weight). About one-third of the walleyes analyzed from the two acidic lakes contained mercury concentrations in excess of the 1.0 µg/g (wet weight) U.S. Food and Drug Administration "Action Level" for human consumption. Given the remote location of the Wisconsin lakes, the close proximity and geologic similarity of the watersheds of the acidic and circumneutral lakes, and the absence of direct anthropogenic inputs of metals to the lakes, these findings suggest that cadmium, lead, and mercury in the naturally acidic lakes are highly available to fish and perhaps other aquatic biota.

Fluctuating asymmetry, defined as deviation from perfect symmetry of any bilateral anatomical character, was previously proposed as an indicator of stress induced by agents affecting calcium metabolism of fish. Fluctuating asymmetry was measured in bluegills and white suckers from selected lakes. Asymmetry of three meristic characters--numbers of pored lateral line scales, pectoral fin rays, and gill rakers on the outer arch--was generally unrelated to lake pH in these species.

These studies indicate that the chemical conditions in the naturally acidic lakes studied here were stressful to certain fish taxa. Species considered to be acid-sensitive were rare or absent in acidic lakes, but were well represented in the circumneutral lakes. Serum calcium measurements suggested that white suckers were stressed in lakes with pH 5.6 or lower. Accumulation of the potentially toxic metals cadmium, lead, and mercury by fish was substantially greater in acidic than in circumneutral lakes.

In studies of susceptible surface waters in Scandinavia and eastern North America, acidification of sensitive surface waters has been detected within one to two decades where precipitation pH was less than 4.6. The pH of precipitation falling in north central Wisconsin is near this apparent threshold value. Consequently, increased loadings of acids to susceptible lakes in north central Wisconsin could increase chronic acid-related stress on fish populations. Piscivorous fishes in acidified waters could accumulate enough mercury in edible muscle tissue to render them unfit for human consumption.

A number of topics for future research are recommended. First, reliable predictions of the potential effects of acidification on these fishery resources will require (1) additional information on the toxicity of low pH to sensitive life stages across the range of waterborne calcium concentrations occurring in susceptible Wisconsin lakes, (2) predictions of the potential mobilization of potentially toxic metals, such as aluminum, into the waters of these lakes during acidification, and (3) dose-response models to quantify water chemistry changes in seepage and drained lakes of this area resulting from acidifying deposition. A second area of recommended research involves the development of indicators of chronic stress associated with early stages of acidification. At present, fishery biologists are lacking *proven* diagnostic tools for detecting stress in fish populations in acidifying lakes before the onset of recruitment failure.

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INTRODUCTION

Acidification of surface waters in Scandinavia and eastern North America has had pronounced adverse effects on fishes and certain other aquatic biota (Haines 1981). Valuable fishery resources have been lost in these regions (Schofield 1976; Wright and Snekvik 1978; Harvey 1980; Overrein et al. 1981; Harvey and Lee 1982), and such losses may become more widespread if acidic precipitation continues. The decline of fish populations in acidified lakes has generally been associated with reproductive failure, rather than with mortality of adult fish (Haines 1981).

Recent investigations of surface water and precipitation chemistry have aroused concern about the potential effects of acidic precipitation on aquatic ecosystems in the north central United States. The mean weighted pH of precipitation at two monitoring stations in north central Wisconsin during 1980 was 4.45 and 4.64 (R. W. Becker, personal communication), or about 10-14 times more acidic than water in equilibrium with atmospheric carbon dioxide, which has a theoretical pH of 5.6 (Vermeulen 1980). Many watersheds in northern Wisconsin have acid soils with low buffering capacity (Hole 1976). The water of most lakes in these watersheds is very soft and slightly acidic. Analyses of 275 northern Wisconsin lakes by Eilers et al. (in press) during 1979 indicated that 36% of the lakes were susceptible to acidification, as judged by the calcite saturation index (Kramer 1976), and that hydrology was the major factor affecting susceptibility. Drained lakes (having no inlet and an intermittent outlet) and seepage lakes (having no inlets or outlets) were most sensitive to acidification because their dissolved mineral content was low and they were influenced little by groundwater inputs. Continuing inputs of acidic precipitation to these poorly buffered, precipitation-dominated lakes could result in a depletion of the buffer system and eventual acidification (Glass et al. 1980).

In this report, I compare selected characteristics of fish populations and communities in two groups of clear-water lakes in north central Wisconsin: (1) six lakes with pHs of 5.1-6.0 (here termed acidic) and (2) six lakes with pHs of 6.7-7.5 (here termed circumneutral). Differences in (1) species composition and richness of fish communities, (2) growth and condition of bluegills, (3) serum calcium concentrations of bluegills and white suckers prior to spawning, (4) accumulation of potentially toxic metals by bluegills and walleyes, and (5) bilateral asymmetry of discrete anatomical characteristics of bluegills and white suckers between the two groups of lakes are statistically compared and discussed in terms of pH-related effects.

Because many water chemistry parameters (e.g., alkalinity, metal concentrations and forms, and calcium concentration) vary concomitantly with pH, it is emphasized that the acidic and circumneutral lakes differed mainly in pH-related chemical characteristics and not solely in pH.

The *central question* addressed by this research project was: Do fishes inhabiting naturally acidic lakes in northern Wisconsin exhibit symptoms of acid/metal stress? If the answer to this question is affirmative, it is likely that acidification of these lakes would cause immediate adverse impacts on fish populations.

Because the data presented herein may be used in future historical comparisons, both the methods and results of this study have been presented in sufficient detail to allow future replication and statistical comparisons. The author anticipates publication of the major findings presented herein in refereed journals and symposia proceedings. Statistical analyses of the data collected during this study is continuing, and results of the analyses in progress will be published in the open literature, as appropriate.

Common names of fishes, standardized by the American Fisheries Society's Committee on Names of Fishes (Robins et al. 1980), are used throughout the text and tables.

DESCRIPTION OF THE STUDY AREA

The lakes studied are located in Oneida County, Vilas County, and the northeastern corner of Lincoln County, Wisconsin (Fig. 1). During the Pleistocene epoch, most of the surface area of these counties was covered by the Chippewa Lobe of the Labrador ice sheet. Most of this area is classified as pitted outwash plain (Hole 1976) and has low relief. Glacial action resulted in the formation of numerous kettle lakes in the area. The Highland Lake District of north central Wisconsin is one of the more concentrated lake districts in the world (Martin 1965). A total of 2.5, 9.5, and 16.8% of the surface areas of Lincoln, Oneida, and Vilas Counties, respectively, are covered by water (Black et al. 1963; Andrews and Threinen 1966; Carlson and Andrews 1982). Most lakes in this area are situated well above the bedrock in thick glacial drift ranging from about 10 to 70 m in thickness (Oakes and Cotter 1975). Soils consist primarily of acidic sands and sandy loams with low cation exchange capacity (Hole 1976). Soils are underlain by Precambrian crystalline rocks, primarily gabbro and basalt, granite and undifferentiated igneous and metamorphic rocks, and quartzite, slate and iron formations (Hole 1976). Elevation ranges from about 450 to 500 m above sea level (Martin 1965).

The climate of this area is cool and humid. Average annual precipitation ranges from about 75 to 80 cm, of which about 60% is lost as evapotranspiration (Hole 1976). Average annual snowfall is about 140 and 200 cm near the southern and northern ends of the study area, respectively. The average length of the annual growing (frost-free)

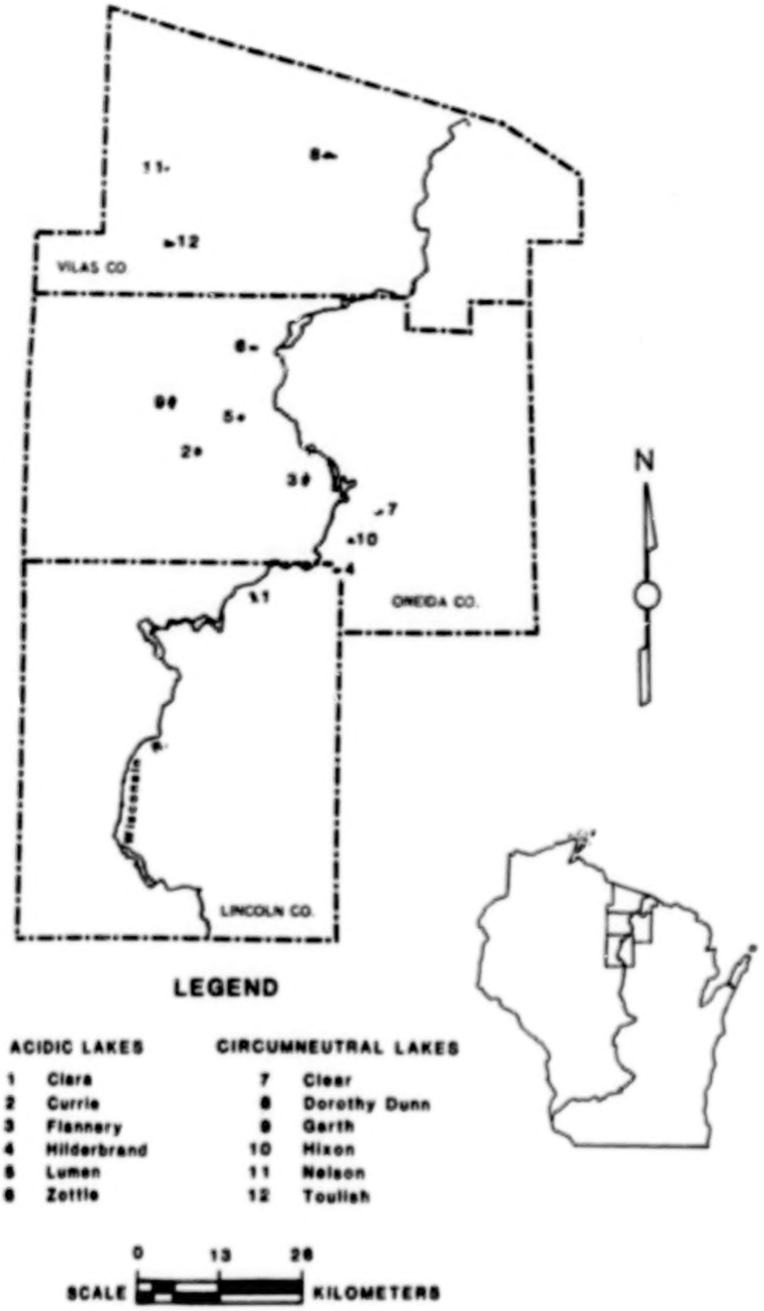

Figure 1. Locations of the 12 study lakes in Lincoln, Oneida, and Vilas Counties, Wisconsin.

season is 100 and 120 days, respectively, at the northern and southern limits of the study area. Average annual temperature is about 4-5 C (Black et al. 1963; Andrews and Threinen 1966).

Upland vegetation was formerly dominated by white pine, which was heavily logged during the late 1800s and early 1900s. The logged upland areas have largely been reforested with deciduous and coniferous species. About 70, 82.5, and 85% of the areas of Lincoln, Oneida, and Vilas Counties, respectively, are now forested (Black et al. 1963; Andrews and Threinen 1966; Carlson and Andrews 1982).

This area is presently receiving acidic precipitation, which is defined here as precipitation having a mean volume-weighted pH of less than 5.0 (Galloway et al. 1982a). The mean weighted pH of event samples of precipitation collected near Rhinelander (Oneida County) during 1980 was 4.45 (R. W. Becker, personal communication). The mean, volume-weighted pHs of precipitation at Trout Lake (Vilas County) during 1980, 1981, and 1982 were 4.64, 4.78, and 4.68, respectively (National Atmospheric Deposition Program data; laboratory measurements by the Illinois Water Survey). Field estimates of mean volume-weighted pH at the Trout Lake NADP station (based on pH measurements made by Wisconsin Department of Natural Resources personnel in Rhinelander) during 1980, 1981, and 1982 were 4.64, 4.65, and 4.58, respectively (R. W. Becker, personal communication). The estimated annual wet deposition of hydrogen ion at Trout Lake during 1980-1982 ranged from 122 to 216 eq/ha for laboratory (Illinois Water Survey) measurements and from 160 to 280 eq/ha for field (Wisconsin Department of Natural Resources) measurements. Annual wet deposition of sulfate and nitrate during the three years ranged 253-377 and 134-196 eq/ha, respectively (R. W. Becker, personal communication).

In studies of susceptible surface waters in Scandinavia and eastern North America, acidification of sensitive surface waters has been detected within one to two decades where precipitation pH was less than 4.6 (Committee on the Atmosphere and the Biosphere 1981:153). Consequently, the pH of precipitation falling in north central Wisconsin is near the threshold pH value considered to cause acidification of sensitive surface waters.

Study lakes were selected to ensure that confounding effects due to differences between the two lake groups in factors such as morphology, hydrologic type, and natural immigration or emigration of fishes were minimized. The following criteria were used for selection of lakes for study:

1. include only seepage and drained lakes;

2. avoid dystrophic lakes (based on water color), winterkill lakes, atypical lakes (e.g., unusually large or deep lakes), and lakes in recently disturbed watersheds; and

3. rank and select 12 lakes according to the availability of historical fisheries and water chemistry data.

Six lakes were naturally acidic and considered susceptible to cultural acidification and six were circumneutral and well buffered (Fig. 1 and Table 1). Ten of the lakes are seepage lakes, and two--Hixon (circumneutral) and Clara (acidic)--are drained by intermittent outlets. One lake (Flannery) is connected to another small seepage lake (not studied) by a permanent channel. Natural migrations of fish to and from

Table 1. Characteristics of Wisconsin lakes in which fish communities were studied.

Lake category and name	Surface area (ha)	Watershed to[a] lake area ratio	SDF[b]	Maximum depth (m)	Mean depth (m)	pH[c]	Alkalinity[c] (ueq/l)	Calcium[d] (mg/l)	Specific[c] conductance (umhos/cm at 25°C)	Secchi disk[e] visibility (m)	Apparent[f] color (Pt-Co units)
Acidic											
Clara	34.1	4.9	1.76	11.4	5.1	6.0	28	1.7	36	4.2	7.5
Currie	39.0	3.8	1.31	4.3	1.9	5.6	6	1.0	16	2.3	20
Flannery[g]	45.3	2.2	2.50	10.6	6.3	5.7	6	1.3	23	3.8	2.5
Hilderbrand	24.2	4.8	1.27	8.0	4.2	5.1	3	1.2	23	3.9	12.5
Lumen	19.8	2.5	1.43	7.2	3.7	5.2	-5	1.0	21	3.9	7.5
Zottle	11.4	5.1	1.48	11.6	4.3	5.6	16	1.0	19	4.3	7.5
Mean	29.0	3.9	1.6	8.9	4.3	5.5	9	1.2	23	3.7	9.6
Range	11.4-45.3	2.2-5.1	1.2-2.5	4.3-11.6	1.9-6.3	5.1-6.0	-5-28	1.0-1.7	16-36	2.3-4.3	2.5-20
Circumneutral											
Clear	14.7	2.8	1.31	7.3	2.4	6.9	400	8.2	98	1.0	10
Dorothy Dunn	28.3	3.0	1.80	8.2	2.7	6.9	312	4.2	39	3.1	7.5
Garth	46.2	2.3	1.20	6.7	3.7	7.5	764	10.1	70	2.4	12.5
Hixon	20.2	7.6	1.82	8.5	3.1	6.8	340	4.7	47	1.5	40
Nelson	10.9	4.7	1.10	15.8	6.7	7.0	342	4.6	42	2.9	7.5
Toulish	32.0	2.3	1.50	11.0	5.5	6.7	402	5.7	47	2.8	10
Mean	25.4	3.8	1.5	9.6	4.0	7.0	427	6.3	57	2.3	14.6
Range	10.9-46.2	2.3-7.6	1.1-1.8	6.7-15.8	2.4-6.7	6.7-7.5	312-764	4.2-10.1	39-98	1.0-3.1	7.5-40

[a] Watershed area included both land and water surface area.

[b] Shoreline development factor.

[c] Means of samples collected at 0.5 m below surface, middepth, and 0.5 m above bottom after the autumnal overturn (October 1981). Mean pH given for each lake was calculated from the mean hydrogen ion concentration.

[d] Mean calcium concentration during summer (see text).

[e] Measured during the October 1981 sampling period.

[f] Samples collected at 0.5 m below surface during June 1982.

[g] Flannery Lake is joined by a channel to another seepage lake (Velvet Lake, 14.2 hectares), which was not sampled.

the twelve lakes are considered to be negligible. The two groups of lakes were morphologically similar, but differed substantially in pH, alkalinity, calcium concentration, and related chemical characteristics (Table 1). The pH values in Table 1 were measured during autumn after the fall overturn and mixing. The pH of northern Wisconsin lakes averages about 0.2 unit lower during autumn than during summer (J. M. Eilers and G. E. Glass, personal communication). The distribution of substrate types did not differ greatly between the naturally acidic and circumneutral lakes. In the acidic lakes, the estimated percent muck, sand, gravel, and rock and rubble ranged from 5-45, 25-70, 10-35, and 0-25, respectively; the percentages were 0-35, 60-75, 5-25, and 0-10, respectively, in the circumneutral lakes (Black et al. 1963; Andrews and Threinen 1966; Carlson and Andrews 1982). Additional descriptions of the study lakes and their watersheds are given in Appendix 1. Morphometric maps of the twelve study lakes, constructed from depth recordings made with a Vexilar recording sonagraph during summer 1980, are shown in Appendices 2-13.

The response of fish to low pH depends partly on external (waterborne) calcium concentrations, and acid toxicity is reduced as waterborne calcium concentration is increased (reviewed by Brown 1982; Wood and McDonald 1982; McDonald 1983). Although calcium data were not collected during the course of the fishery investigations reported here, the author has since cooperated with graduate students (Penny S. Schmidt and David E. Powell) and staff (Ronald G. Rada) of the University of Wisconsin-La Crosse to quantify concentrations of major ions and selected trace metals in these and other northern Wisconsin lakes. This effort is still ongoing, and results will be summarized in theses and published reports. However, I have summarized calcium concentrations measured in the twelve study lakes (near-surface samples) during five sampling periods from June 1982 to June 1983 (Appendix 14). Analytical precision during these calcium determinations averaged 0.5% (relative standard deviation, RSD), and the mean percent recovery from spiked samples was 93%. Variability among replicate samples from the field averaged 7.0% (RSD). Calcium concentrations during summer 1979, measured in seven of these lakes by J. M. Eilers and G. E. Glass, are also tabulated in Appendix 14.

A number of conclusions can be drawn from the calcium data collected to date. First, the calcium values measured during summer 1979 are similar to, or fall within the range of, concentrations measured during the summers of 1982-1983 (data for June and August; Appendix 14). Therefore, statistical analysis of the 1982-1983 calcium data in conjunction with the fishery data from 1980-1981, presented herein, is valid. Second, the calcium concentrations in near-surface waters of all six naturally acidic lakes were less during summer than after the autumnal overturn (October samples) or during winter (January samples); this trend was not apparent in the circumneutral lakes. Consequently, I have elected to use the mean calcium concentrations measured during summer (tabulated in Table 1 and Appendix 14) in statistical tests. Third, calcium concentrations were substantially less in the naturally acidic lakes than in the circumneutral lakes. At calcium concentrations less than 2.0 mg/l, waterborne calcium can significantly influence a fishery's response to acid stress (Brown

1982). Mean calcium concentrations during summer were less than 2.0 mg/l in all six acidic lakes (Table 1 and Appendix 14).

Chancey Juday, E. A. Birge, and their co-workers at the Wisconsin Geological and Natural History Survey conducted limnological surveys of lakes in the Highland Lake District of Wisconsin during the 1920s and 1930s (Frey 1963). The occurrence of numerous naturally acidic lakes in northern Wisconsin was documented nearly 50 years ago by Juday et al. (1935). Bowser et al. (unpublished) have compared the current pH, alkalinity, and conductivity of a number of northern Wisconsin lakes to values recorded for those lakes half a century ago by Birge, Juday, and their co-workers and found no evidence of recent cultural acidification. On the basis of their findings, it is assumed that the acidic lakes studied here are naturally acidic.

HISTORICAL RECORDS OF FISH POPULATONS, FISH STOCKING, AND WATER CHEMISTRY

Historical records of fish populations and fish stocking were obtained from Wisconsin Department of Natural Resources lake files in Antigo, Rhinelander, and Woodruff. The file for one of the twelve study lakes (Zottle Lake) could not be located and is still missing.

Information from previous fishery surveys were obtained for six of the lakes studied--three acidic (Hilderbrand, Flannery, and Clara Lakes) and three circumneutral (Dorothy Dunn, Nelson, and Garth Lakes). Dates of these surveys ranged from 1944 to 1969 (Appendix 15). These surveys were generally conducted with a single type of sampling gear (fyke net, gill net, large seine, or electroshocker) for a period of one to four days during spring or summer. Species recorded during these surveys consisted primarily of esocids (northern pike and muskellunge), ictalurids (black and yellow bullhead), centrarchids (rock bass, pumpkinseed, bluegill, smallmouth bass, largemouth bass, black crappie, and warmouth), and percids (yellow perch and walleye). Either the fishing gear employed was inappropriate for collecting smaller bodied fishes such as minnows, the occurrence of such species in the catch was not recorded, or such species were not present.

Records from Wisconsin Department of Natural Resources files indicated that fish had been stocked into eight of the twelve lakes studied--four acidic (Clara, Currie, Flannery, and Hilderbrand Lakes) and four circumneutral (Dorothy Dunn, Garth, Nelson, and Toulish Lakes)--as of summer 1980. The files for Clear, Hixon, and Lumen Lakes contained no records of fish stocking. Dates of fish stocking ranged from 1937 to 1980 (Appendix 16). During the early 1940s, fishes were commonly collected from other drainage systems, transported, and stocked into northern Wisconsin lakes (L. M. Andrews, personal communication). At that time, fishes stocked into a lake were commonly recorded as "shiners", "chubs", and "suckers"; the actual species of fish included in these groupings are unknown. The species most commonly stocked into these lakes were game fishes, primarily northern pike, muskellunge, largemouth bass, and walleye (Appendix 16). Contacts with the public suggest that accidental and intentional releases of fish (particularly bait minnows) into these lakes by fishermen, which is illegal under Wisconsin law without approval by the Department of Natural Resources, have probably been commonplace during recent years. Differences between recent (1980-1981) species composition of fish communities and historical fisheries (stocking and survey) data on these lakes are discussed in the following chapter.

Historical water chemistry data, collected during the 1920s and 1930s by Chancey Juday, E. A. Birge, and their co-workers at the Wisconsin Geological and Natural History Survey, are available for six of these lakes--one acidic and five circumneutral. These data, which are primarily from analyses of near surface samples taken during summer, are given in Appendix 17.

SPECIES COMPOSITION OF FISH COMMUNITIES IN ACIDIC AND CIRCUMNEUTRAL NORTHERN WISCONSIN LAKES*

The species structure and composition of fish communities in lakes is affected by an array of physical, chemical, biological, and geographic factors (Barbour and Brown 1974; Emery 1978; Tonn and Magnuson 1982; Tonn et al. 1983). Studies of culturally acidified lakes of the northeastern United States, eastern Canada, and Scandinavia indicate that changes in pH and related chemical factors strongly impact the fish communities of these waters (Harvey 1975; Beamish et al. 1975; Wright and Snekvik 1978; Haines 1981; Mills 1983). During the acidification process, lacustrine fish communities decline in species richness as the more acid-sensitive species populations become extinct. Eventually, species considered to be acid tolerant may decline in numbers or disappear.

In this chapter, the species composition and richness of fish communities in naturally acidic and circumneutral clear-water lakes in northern Wisconsin are compared. Confounding effects due to differences between the two lake groups in morphology, hydrologic type, and immigration and emigration of fishes were minimized by the lake selection process. Differences in the species richness and composition of fish communities between the two groups of lakes are statistically compared and discussed in terms of pH-related effects.

Methods

Comprehensive sampling of fish communities was begun on 8 July and completed on 20 August 1980. Fish were collected with gill nets, fyke nets, minnow traps, and seines. A standard sampling protocol was followed and yielded equal sampling effort on each lake, with only minor exceptions (Table 2). Locations of fyke net sets, gill net sets, and shoreline seining in each lake are shown in Appendices 2-13. Minnow traps were located near fyke net sets. Minnows were identified according to Becker and Johnson (1970). All fish were measured (total length) to the nearest mm, with one exception--approximately half of the 8,624 bluegill collected from Lumen Lake were not measured.

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*A modified version of this chapter, co-authored by Paul J. Rago and Joseph M. Eilers, is in press (Wiener et al. 1983).

Table 2. Summary of sampling effort for characterizing fish communities in study lakes.

Type of gear	Effort per lake
300-ft (91.4 m), variable mesh gill net	4 nets for 2 nights*
Small mesh (3/8 inch or 0.95 cm) fyke net	7 nets for 2 nights*
Minnow trap (1/4-inch or 0.64-cm mesh)	10 traps for 2 nights*
25-ft (7.6 m) bag seine with 3/16 inch (0.48 cm) mesh in bag	25 seine hauls

*Fish removed daily.

Approximately 50 fish of each species from each lake, or the number caught if less than 50, were weighed to the nearest 0.1 g.

Water samples were collected during the fish sampling period and analyzed for pH and alkalinity; however, later analyses indicated that pH readings obtained with the field meter being used at that time were inaccurate. Subsequent water sampling was conducted on all 12 lakes during October 1981, after the autumnal overturn, when lakes were completely mixed. Water samples from each lake were collected near the point of maximum depth at 0.5 m below surface, middepth, and 0.5 m above bottom. Differences in water temperature between points 0.5 m below surface and 0.5 m above bottom for the 12 lakes averaged 0.2 C and ranged from 0.0 to 0.5 C during this sampling period. The pH of the October 1981 samples was measured with an Orion model 407A meter, total alkalinity by Gran titration (Stumm and Morgan 1981), and specific conductance with a YSI model 32 conductivity meter. Mean pH of each lake was calculated as the negative logarithm of the average hydrogen ion concentration over depths. Apparent color (unfiltered water samples) of water samples collected at 0.5 m below surface during June 1982 was measured with a Hellige model 611-A Aqua Tester. Apparent and true color of water from northern Wisconsin lakes are similar (Eilers et al. in press). Data analyses were conducted with the Michigan Interactive Data Analysis System (MIDAS) at the University of Michigan, Ann Arbor (Statistical Research Laboratory 1976).

Results and Discussion

Summer 1980 Fishery Survey

Results of chemical analyses of lake water have been given in Table 1. A total of 30,388 fish, representing 27 species and 10 families (Appendix 18), were collected during the fishery survey of the 12 lakes. The number of fish collected from individual lakes ranged from 1,141 to 9,234 (Table 3). Numbers of fish collected by individual gear types in each lake are given in Appendix 19. Fish communities in the lakes were dominated largely by members of the sunfish family (Centrarchidae) and perch family (Percidae; Table 4). Only two species, largemouth bass and yellow perch, were found in all 12 lakes (Table 5).

Species richness (number of fish species) was lower in the naturally acidic lakes than in the circumneutral lakes. A total of 15 species were collected in the six acidic lakes, and 25 species were found in the circumneutral lakes (Tables 4 and 5). Species richness ranged from 5 to 11 in the acidic lakes and from 10 to 17 in the circumneutral lakes (Table 3). A Mann-Whitney test indicated that species richness was significantly lower in acidic lakes than in circumneutral lakes ($P < 0.025$). Similarly, the Pearson correlation coefficient between species richness and lake pH revealed a positive relation between the two variables ($r = 0.68$, $P < 0.05$). These results are in contrast with those of Rahel and Magnuson (1980), who studied fish communities in 19 small seepage, nonwinterkill dystrophic lakes in northern Wisconsin; their study lakes were physically similar but ranged in pH from 4.3 to 6.9, and no relation was found between species richness and pH. More extensive analyses by Rahel (1982) indicated that species richness in bog lakes is limited by habitat characteristics other than pH, such as depth, substrate type, and dissolved oxygen concentration.

Minnows and darters, which are generally relatively sensitive to low pH (Harvey 1980), were either absent or rare in acidic lakes, but were generally well represented in the circumneutral lakes (Table 5). Only 5 minnows (two species) were found in the acidic lakes, whereas a total of 4,231 minnows, representing six species, were found in the circumneutral lakes. Minnows were collected in only two acidic lakes, but were collected from all six circumneutral lakes (Table 3). The bluntnose minnow, an acid-sensitive species (Harvey 1980; Rahel and Magnuson 1983), occurred in five of the circumneutral lakes and was the most abundant cyprinid (Table 5). The only minnow species found in more than one of the acidic lakes (four individuals in two lakes) was the golden shiner, which is more acid-tolerant than the other minnow species collected (Harvey 1980; Rahel and Magnuson 1983). In a survey of fish communities in northern Wisconsin lakes, Rahel and Magnuson (1983) found that no cyprinid species occurred at pHs below 5.2 and that darters and most cyprinids were absent at pHs below 6.2. Four of the circumneutral lakes in the present study contained darters, but none were found in the acidic lakes (Table 3).

Table 3. Numbers of fish of different species collected during comprehensive sampling of fish communities in 12 northern Wisconsin lakes (all gear types combined).

Species	Acidic lakes (pH shown in parentheses)						Circumneutral lakes (pH shown in parentheses)					
	Hilderbrand (5.1)	Lumen (5.2)	Currie (5.6)	Zottle (5.6)	Flannery (5.7)	Clara (6.0)	Toulish (6.7)	Hixon (6.8)	Clear (6.9)	Dorothy Dunn (6.9)	Nelson (7.0)	Garth (7.5)
Cisco	0	0	0	0	0	0	0	0	0	0	26	0
Central mudminnow	0	0	0	0	0	0	0	0	0	1	1	0
Northern pike	11	0	0	0	1	20	18	1	0	0	0	7
Muskellunge	0	0	0	0	0	0	0	0	0	11	0	0
Golden shiner	0	2	2	0	0	0	0	18	0	9	0	8
Blackchin shiner	0	0	0	0	0	0	0	0	0	0	20	0
Blacknose shiner	0	0	0	0	0	0	32	0	0	0	129	0
Emerald shiner	0	0	0	0	0	0	0	0	0	96	0	0
Weed shiner	0	0	0	0	0	0	0	0	0	0	0	6
Bluntnose minnow	0	0	0	0	0	0	354	264	1,185	0	1,032	1,078
Creek chub	0	0	1	0	0	0	0	0	0	0	0	0
White sucker	6	1	217	15	2	0	2	4	44	17	12	0
Black bullhead	8	7	0	0	0	0	0	0	9	0	0	5
Yellow bullhead	0	0	0	0	116	178	4	91	19	2	1	70
Brook stickleback	0	0	0	0	0	0	0	0	0	0	1	0
Rock bass	0	118	9	303	0	0	45	2	0	0	153	2
Green sunfish	0	0	34	0	52	0	0	0	0	0	0	0
Pumpkinseed	524	0	42	193	786	0	20	388	5	0	188	474
Bluegill	39	8,624	176	0	474	792	636	816	171	127	981	1,912
Smallmouth bass	0	38	0	0	1	0	4	0	0	0	2	1
Largemouth bass	43	145	119	56	8	44	58	65	23	183	45	112
Black crappie	0	0	68	0	1	0	35	85	0	0	4	103
Iowa darter	0	0	0	0	0	0	0	0	0	0	5	0
Johnny darter	0	0	0	0	0	0	1	9	6	0	0	0
Yellow perch	969	299	737	747	544	285	38	159	210	681	104	1,030
Walleye	1	0	12	0	1	23	0	1	9	14	8	1
Mottled sculpin	0	0	0	0	0	0	1	0	0	0	0	0
Total number of fish	1,601	9,234	1,417	1,314	1,986	1,342	1,248	1,903	1,681	1,141	2,712	4,809
Total number of species	8	8	11	5	11	6	14	13	10	10	17	14

Table 4. Total number of fish of different families collected during sampling of six acidic and six circumneutral lakes in northern Wisconsin. Number of species represented in each family is given in parentheses.

Family	Lake category	
	Acidic	Circumneutral
Salmonidae	0	26 (1)
Umbridae	0	2 (1)
Esocidae	32 (1)	37 (2)
Cyprinidae	5 (2)	4,231 (6)
Catostomidae	241 (1)	79 (1)
Ictaluridae	309 (2)	201 (2)
Gasterosteidae	0	1 (1)
Centrarchidae	12,689 (7)	6,640 (6)
Percidae	3,618 (2)	2,276 (4)
Cottidae	0	1 (1)
Total	16,894 (15)	13,494 (25)

The number of fish species other than darters and minnows ranged from 5 to 11 in the acidic lakes and from 8 to 13 in the circumneutral lakes. A second Mann-Whitney test of species richness of fishes other than darters and minnows was conducted to determine if differences in species richness between the two groups of lakes were related primarily to the scarcity of darters and minnows in the acidic lakes. Species richness of fishes other than darters and minnows was also significantly less in the acidic than in the circumneutral lakes ($P < 0.05$).

The ranges of waterborne calcium concentrations did not overlap between the naturally acidic and circumneutral lakes (Appendix 14). Furthermore, mean calcium concentrations during summer were 2.5- to 10-fold higher in the individual circumneutral lakes than in the individual acidic lakes. Fish species richness in the twelve lakes was significantly (rank) correlated with mean calcium concentration during summer ($r_s = 0.63$, $0.01 < P < 0.025$; one-tailed test for positive correlation). Consequently, it is not feasible to distinguish between the effects of pH and calcium on the composition and richness of these fish communities with existing data. At concentrations less than 2 mg/l, calcium can significantly influence survival times of fish experiencing acute acid stress in the laboratory (Brown 1982). The status of fish populations in acidified Norwegian lakes is also related to waterborne calcium concentration (Wright and Snekvik 1978). Consequently, the differences in species richness and composition of fish communities between the naturally acidic and circumneutral lakes studied here were probably due to two confounded factors, low pH in combination with low waterborne calcium concentration.

No relation between species richness and lake area was apparent for the lakes studied here, probably because I selected lakes within a

Table 5. Comparison of total numbers of each species caught in acidic and circumneutral lakes. The number of lakes in which each species was found is indicated in parentheses after each total.

Species	Acidic lakes (n=6)	Circumneutral lakes (n=6)
Cisco	0	26 (1)
Central mudminnow	0	2 (2)
Northern pike	32 (3)	26 (3)
Muskellunge	0	11 (1)
Golden shiner	4 (2)	35 (3)
Blackchin shiner	0	20 (1)
Blacknose shiner	0	161 (2)
Emerald shiner	0	96 (1)
Weed shiner	0	6 (1)
Bluntnose minnow	0	3,913 (5)
Creek chub	1 (1)	0
White sucker	241 (5)	79 (5)
Black bullhead	15 (2)	14 (2)
Yellow bullhead	294 (2)	187 (6)
Brook stickleback	0	1 (1)
Rock bass	430 (3)	202 (4)
Green sunfish	86 (2)	0
Pumpkinseed	1,545 (4)	1,075 (5)
Bluegill	10,105 (5)	4,643 (6)
Smallmouth bass	39 (2)	7 (3)
Largemouth bass	415 (6)	486 (6)
Black crappie	69 (2)	227 (4)
Iowa darter	0	5 (1)
Johnny darter	0	16 (3)
Yellow perch	3,581 (6)	2,222 (6)
Walleye	37 (4)	33 (5)
Mottled sculpin	0	1 (1)

narrow size range and the number of lakes surveyed was not large (Table 1). Pearson correlation coefficients between species richness and lake area, calculated for all lakes and for circumneutral lakes, were nonsignificant. However, a marginally significant correlation (r = 0.77, $0.05 < P < 0.10$) between lake area and species richness was observed for the six acidic lakes. Analysis of covariance of species richness between lake groups with lake area as a covariate yielded similar results; differences in mean species richness between acidic and circumneutral lakes were highly significant ($P < 0.005$), and lake area was not a significant factor (P ~0.21). Bounded plots of species richness against lake area showed virtually no overlap between acidic and circumneutral lakes (Fig. 2).

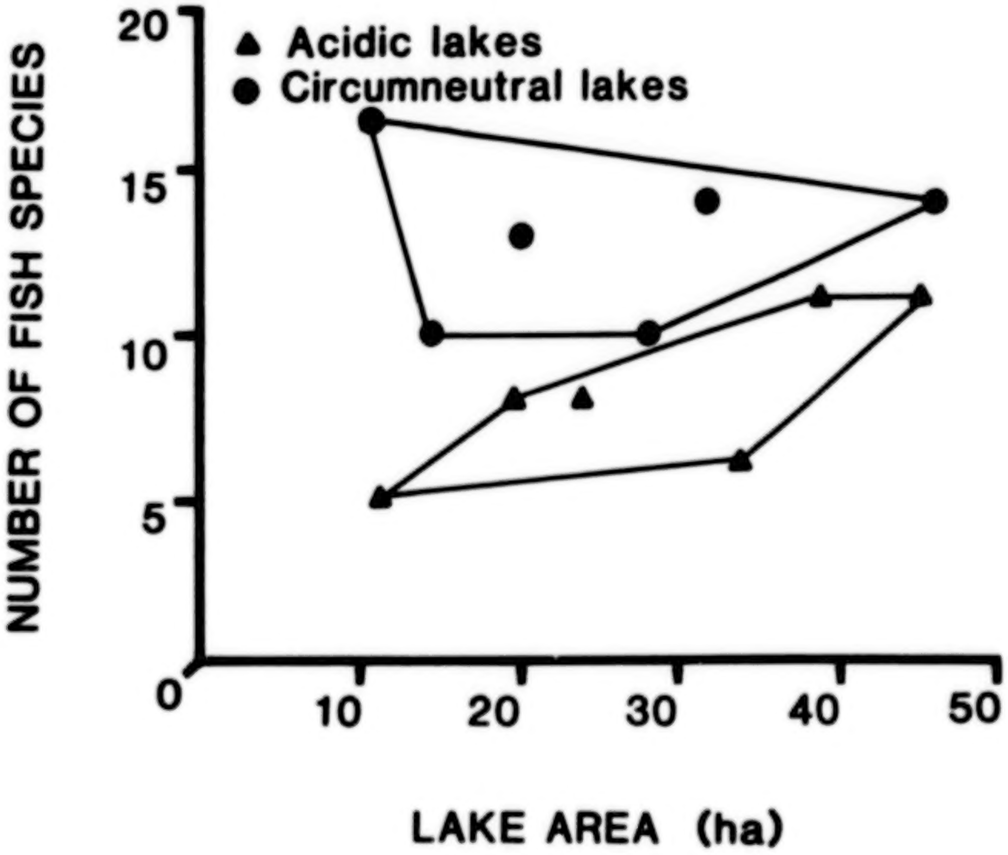

Figure 2. Relation between number of fish species and surface area of acidic and circumneutral lakes in north central Wisconsin. Points for each lake group were bounded to show separation between the two groups.

The occurrence of a fish species in one or two lakes of a given lake group could be attributed to chance rather than to suitability of the aquatic habitat for that species. Numbers of fish species in acidic and circumneutral lakes were therefore compared according to frequency of occurrence within each lake group. Results of this comparison (Fig. 3) indicated that common as well as rare species occurred less frequently in the naturally acidic lakes than in the circumneutral ones.

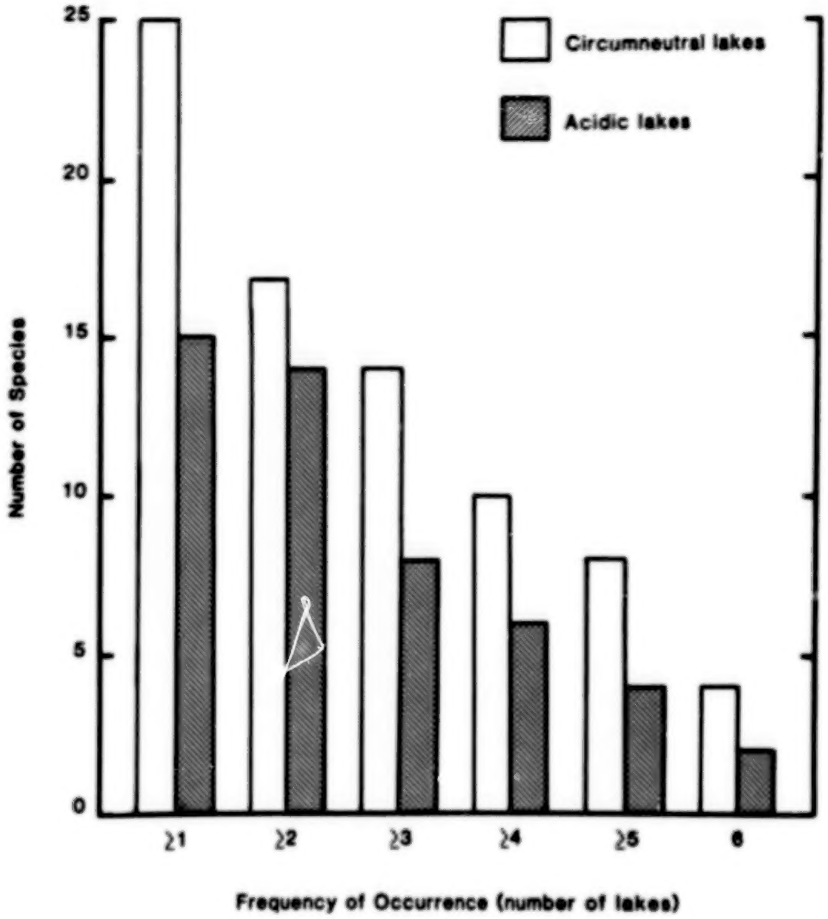

Figure 3. Number of fish species in the acidic and circumneutral lake groups as a function of frequency of occurrence in each group.

For example, only 8 species were found in three or more acidic lakes, whereas 14 species were found in three or more circumneutral lakes.

A total of 38 smallmouth bass were collected from Lumen Lake (Table 3), which had an autumnal pH of 5.2. Total lengths, measured for 21 of these fish, ranged from 96 to 228 mm (median, 119 mm). Externally visible deformities were not recorded for any of these specimens. The smallmouth bass is considered an acid-sensitive species, and reportedly is adversely affected at pH values of 5.5 to 6.0 or lower (Table 3 in Haines 1981). However, most investigators report pH values measured during summer, which are not directly comparable with the lake pHs given in Table 1. The pH of northern Wisconsin lakes averages about 0.2 unit lower during autumn than during summer (J. M. Eilers and G. E. Glass, personal communication). The pH of surface water from Lumen Lake during summer 1979 was 5.5, suggesting that the smallmouth bass population in Lumen Lake may be near the lower range of pH tolerance for the species. An apparently healthy smallmouth bass population has also been observed in Sugar Camp Lake in north central Wisconsin, which has a summer pH of 5.3 (L. M. Andrews, personal communication; J. M. Eilers and G. E. Glass, personal communication).

In a comparative field study of this type, it is not possible to state unequivocally that observed patterns are due solely to a single factor or group of related factors--in the present situation, water chemistry. There is, however, strong evidence suggesting that certain species should not occur in certain lakes, e.g., those in which pH is below the presumed physiological tolerance range for the species in question (Harvey 1980; Haines 1981).

It is unlikely that the two groups of lakes studied here have dissimilar geologic histories relative to connection to other water bodies and consequently, to past migrations and invasions of fishes. All of the study lakes are glacial in origin from the same glacial lobe (USGS 1976). The acidic and circumneutral lakes are not greatly dissimilar with respect to glacial till, and the proximity of some of the acidic and circumneutral lakes (Fig. 1) reduces the likelihood that the differences in fish communities between the two groups of lakes are due to some spatial factor.

It is considered unlikely that the differences in fish community composition between the two groups of lakes are related to productivity. Limited data on chlorophyll *a* concentrations, obtained during the summer 1979 survey of Eilers et al. (in press) and used here as an index of primary productivity, suggests that primary productivity does not differ greatly between the acidic and circumneutral lakes in this study. Chlorophyll *a* in 2-m integrated surface water samples ranged from 1.84 to 3.91 µg/l (average, 3.0 µg/l) in the six acidic lakes and were 8.74 and 2.74 µg/l, respectively, in two of the circumneutral lakes (Garth and Nelson Lakes).

The differences in the presence and abundance of cyprinids between the acidic and circumneutral lakes (Table 4) are striking. The bluntnose minnow, the most abundant cyprinid encountered (Table 5), is a prolific spawner (Gale 1983) and should be capable of rapidly exploiting new habitats if environmental conditions are favorable. Minnows may be introduced into lakes in this area by fishermen releasing bait fishes at day's end. There is no apparent reason why such introductions of minnows should be more frequent in the circumneutral than in the acidic lakes. In addition, no major differences in the presence of predatory fishes (northern pike, muskellunge, smallmouth and largemouth bass, black crappie, and walleye) are readily apparent between the acidic and circumneutral lakes (Table 3). It is therefore concluded that the acidic lakes are unsuitable for most minnow species because of water chemistry factors, probably pH and calcium. This assertion is supported by published information on pH levels at which cyprinid species were adversely affected in both laboratory experiments and natural waters (Tables 3 and 4 in Haines 1981).

Other Fish Collections

Additional fish sampling has been conducted by the Field Research Station staff on ten of the twelve study lakes since the comprehensive (summer 1980) survey (Table 6). Except for three lakes sampled by

Table 6. Summary of fish sampling conducted on the study lakes since the summer 1980 survey and additional fish species recorded during these sampling events.

Lake category and name	Sampling date and gear type			Additional[a] species recorded
	October 1980 (electroshocker)	May 1981 (fyke net)	June 1981 (fyke net)	
Acidic				
Lumen			X	None
Currie		X	X	Black bullhead
Zottle		X		None
Flannery			X	Muskellunge
Clara			X	Pumpkinseed Black Crappie
Circumneutral				
Toulish			X	Muskellunge
Hixon			X	None
Dorothy Dunn	X			None
Nelson	X	X	X	Muskellunge Golden shiner Common shiner[b]
Garth	X		X	None

[a] Species not collected during the summer 1980 survey.

[b] *Notropis cornutus*.

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electroshocking during late October 1980, these later collections were made with fyke nets to obtain white suckers (May 1981 sampling) and bluegills (June 1981 sampling) for serum calcium analysis. No fish sampling has been conducted by us on Hilderbrand (acidic) and Clear (circumneutral) Lakes since the summer 1980 survey.

No additional species of fish (i.e., species not collected during the summer 1980 survey) were found in five of these lakes during these subsequent samplings (Table 6). One additional fish species was collected in each of three lakes, and two and three additional species were collected in a single lake each. In most cases, the additional species recorded for a given lake during these sampling events were represented by only one or two individuals and were presumably scarce. Inclusion of these additional species for each lake (from Table 6) into the total number of species for each lake on Table 3 does not alter the conclusions derived from the summer 1980 survey data; species richness remained significantly less in the acidic than in the circumneutral lakes (Mann-Whitney test, $0.01 < P < 0.025$).

Most of the fish species present during earlier fishery surveys of six of these lakes (Appendix 15) were found during our 1980-1981 sampling. Fishes recorded as present during previous surveys but not found during 1980-1981 were: (1) warmouth and black bullhead in Flannery Lake, surveyed during 1967, (2) white sucker in Clara Lake, surveyed during 1969, and (3) white sucker in Garth Lake, surveyed during 1950.

Similarly, most of the fish species stocked into these lakes (Appendix 16), excluding "shiners", "chubs", and "suckers", were present during our 1980-1981 fish sampling. Species stocked but not found in a given lake included: (1) rainbow trout in Flannery Lake, (2) fathead minnow in Clara Lake, (3) northern pike in Dorothy Dunn Lake, and (4) muskellunge in Garth Lake. The absence of certain previously stocked species in our catch is not surprising. For example, survival of stocked muskellunge and northern pike in Wisconsin lakes is often as low as 0-1% (Johnson 1978).

Summary

Fish communities in naturally acidic Wisconsin lakes (pH 5.1-6.0) contained significantly fewer species than did those in circumneutral lakes. Common as well as rare species occurred with lower frequency in acidic lakes than in circumneutral lakes. Certain taxa, such as minnows and darters, were well represented in circumneutral lakes but were either absent or rare in the acidic lakes, probably because of pH-related stress and low waterborne calcium concentrations. Species richness of fishes other than minnows and darters was also significantly lower in acidic than in circumneutral lakes. The differences in species composition and richness of fish communities between acidic and circumneutral lakes did not appear to be related to differences in physical habitat characteristics, past fish migrations, or productivity between the two lake groups. Water chemistry characteristics, probably pH and waterborne calcium, strongly influenced the species composition and richness of fish communities in these lakes.

GROWTH AND CONDITION OF BLUEGILLS IN RELATION TO LAKE pH AND POPULATION DENSITY*

In this chapter, the effects of two environmental factors, pH and population density, on condition and growth of bluegills in eleven of the study lakes are reported. The applicability of data on condition and growth for assessing pH-related stress on bluegill populations is evaluated.

In the laboratory, fish exposed to low pH commonly exhibit reduced growth. The growth rates of fry of flagfish *Jordanella floridae* fed ad libitum during flow-through bioassays were similar at pH levels of 6.8 and 6.0, but were considerably lower at pH 5.5 and 5.0 (Craig and Baksi 1977). Growth of flagfish fry was also significantly less at pH 5.0 than at pH 5.5. Similar reductions in growth of alevins of brook trout *Salvelinus fontinalis* in response to decreasing pH in continuous-flow systems were reported by Menendez (1976); growth of the alevins was significantly less at pH 5.5, 6.0, and 6.5 than at pH 7.1. Edwards and Hjeldnes (1977) found that growth rates of yearling rainbow trout *Salmo gairdneri* and Arctic char *Salvelinus alpinus* fed ad libitum in the laboratory were less at pH 4.8-5.0 than at pH 5.5 and 6.1-6.2. Nelson (1982) reported that growth (length) of laboratory-reared alevins of rainbow trout was significantly less at pH 4.3, 4.6, and 4.8 than at pH 7.1 and 7.3. Growth of alevins at the pHs tested was not affected by waterborne calcium concentration, which ranged from 5.5 to 112 mg/l in Nelson's study.

In field studies, investigators have reported both increased and decreased rates of growth within a single fish species in acidified waters (Baker 1983). Beamish (1974) found that growth rates of white suckers were lower in acidified Lumsden Lake (Ontario) after a reduction in pH from 6.8 to 4.6. After acidification of lakes of the La Cloche Mountains, Ontario, the growth rates of yellow perch of age groups 1-3 increased, whereas those of age groups 4-9 declined (Ryan and Harvey 1980). The increase in growth rates of the younger yellow perch after acidification of these lakes may have resulted from reduced competition for food due to declining recruitment (Ryan and Harvey 1980).

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*A modified version of this chapter, co-authored by Wendy R. Hanneman, has been published (Wiener and Hanneman 1982).

Results of the above studies suggest that the effects of acidification on growth of fishes in natural waters is confounded by changes in fish density or food availability. The relative effects of pH and population density on the growth rates and condition factors of bluegills in Wisconsin lakes are reported here. Specific objectives were to (1) compare growth and condition factors of bluegills in clear-water, northern Wisconsin lakes with acidic and circumneutral pH values and (2) evaluate suitability of condition factors and growth rates of bluegills as indicators of chronic, pH-related stress on bluegill populations. Many aquatic resource agencies maintain extensive historical data files on fish growth rates in public waters. From this standpoint, the application of data on fish growth to assess pH-related stress on fish populations resulting from cultural acidification of surface waters is conceptually attractive. Bluegills were selected as the study organism because they occur extensively in northern Wisconsin lakes and were found in eleven of the twelve study lakes.

Methods

Fish were collected from 8 July to 20 August 1980, during the comprehensive survey of fish communities. Bluegills used in this analysis were collected with 91.4-m, variable-mesh gill nets and 0.95-cm mesh fyke nets. Seven fyke nets and four gill nets were set in each lake for two days and nights and checked daily. This sampling protocol was followed on each lake (sampling effort was equal), with only one exception; three gill nets, rather than four, were used on Flannery Lake. After collection, bluegills were placed into plastic bags and held in ice-filled chests at the sampling site. Samples were frozen within 12 hours after capture and stored at about -4 C until measurement. Each fish was weighed to the nearest 0.1 g and measured (total length) to the nearest millimeter. Scales for age determinations were taken immediately posterior to the point of insertion of the left pectoral fin. Scale impressions were made and examined at a magnification of 38X on an Eberbach scale reader. Age assigned to each fish was equal to the total number of completed scale annuli, according to criteria described by Tesch (1971). Back-calculated total lengths of bluegills were estimated by the method of Whitney and Carlander (1956). Condition factors (K) of bluegills were calculated with the formula, $K = W/L^3$, where W is wet weight (g) and L is total length (mm).

Data analyses were conducted with the Michigan Interactive Data Analyses System (MIDAS) at the University of Michigan, Ann Arbor (Statistical Research Laboratory 1976). Statistical significance is defined as $P \leq 0.05$.

Results and Discussion

Most (98%) of the bluegills obtained for growth analysis were captured in fyke nets (Table 7). Catch in fyke nets alone or combined

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Table 7. Numbers of bluegills collected and ranges of age and size of bluegills analyzed from each lake studied.

Lake	Sampling dates (1980)	Number caught		Number analyzed	Range in age (years)	Range in total length (mm)	Range in wet weight (g)
		Fyke nets	Gill nets				
			Acidic lakes				
Clara	August 4-5	569	10	47	3-7	107-178	17.8- 98.2
Currie	July 16-17	172	0	50	1-12	73-235	6.4-261.6
Flannery	July 8-10	420	5	56	2-8	91-180	11.1-107.8
Hilderbrand	July 21-22	39	0	33	3-7	106-201	19.7-145.5
Lumen	July 14-15	8,481	47	50	5-10	142-212	44.9-160.0
			Circumneutral lakes				
Clear	July 30-31	122	3	49	2-8	86-205	9.1-191.1
Dorothy Dunn	July 28-29	16	0	16	1-7	84-201	8.2-161.3
Garth	August 6-7	1,671	163	47	3-7	93-155	11.9- 61.4
Hixon	July 23-24	655	23	51	1-9	55-163	2.7- 78.7
Nelson	August 18-19	576	12	51	1-8	75-163	4.7- 62.0
Toulish	August 13-14	452	12	48	1-11	75-215	6.6-185.3

catch in fyke and gill nets provided equivalent rankings of individual lakes with regard to relative density of bluegill populations.

Condition factors of bluegills varied significantly among lakes ($P < 0.001$; one-way analysis of variance), but these differences were unrelated to lake pH. A Mann-Whitney test of mean K showed no significant difference between acidic and circumneutral lakes, and the Spearman (rank) correlation coefficient between mean K and lake pH was also insignificant. Multiple comparison procedures indicated relatively little overlap of K among lakes, and no consistent patterns for acidic and circumneutral lakes (Table 8). Rather, there was a strong inverse relation between mean condition factor and relative abundance of bluegills (Table 8). The Spearman correlation coefficient between mean K and total catch of bluegills was -0.93 ($P < 0.001$).

Table 8. Mean condition factors (K = weight/length^3) and relative density (number of individuals) of bluegills from eleven lakes in northern Wisconsin, listed in order of decreasing condition factors. Means with no letters in common differ significantly ($P < 0.05$; Scheffe's multiple comparison of means).

Lake[a]	Rank of density[b]	Mean condition factor ($\times 10^5$)
Dorothy Dunn	11	2.09 a
Hilderbrand	10	1.75 b
Clear	9	1.73 b,c
Currie	8	1.72 b,c
Flannery	7	1.67 c,d
Toulish	6	1.64 d,e
Hixon	3	1.59 e
Clara	5	1.57 e,f
Garth	2	1.51 f,g
Lumen	1	1.44 g
Nelson	4	1.37 h

[a] Names of naturally acidic lakes (pH 5.1-6.0) are italicized.

[b] Based on number of bluegills caught in fyke nets (Table 7); rank of 1 is highest density, and rank of 11 is lowest density.

Growth rates of bluegills from Lumen (acidic) and Nelson (circumneutral) lakes were extremely slow, making distinctions between annuli uncertain. Therefore, samples from these lakes were not included in statistical analyses of back-calculated lengths.

Back-calculated total lengths of bluegills at ages 1, 2, 3, and 4 (Table 9) varied significantly among lakes ($P < 0.001$; one-way analysis of variance). However, Mann-Whitney tests of mean back-calculated lengths revealed no significant difference between acidic and circumneutral lakes for any of the four age groups, even at the 10% level of significance. In contrast, an inverse relation between growth rate and relative density of bluegills was apparent. Spearman correlation coefficients (r_s) between relative density and mean back-calculated total length of bluegills (one-tailed test for negative correlation) were not significant at age 1 ($r_s = 0.13$), but were significant at ages 2 ($r_s = -0.59$; $P < 0.05$), 3 ($r_s = -0.63$; $P < 0.05$), and 4 ($r_s = -0.90$; $P < 0.01$). The strength of the negative correlation between ranks of density and mean length increased with age.

These results indicate that growth and condition of bluegills in northern Wisconsin seepage lakes were related to density and not to lake pH. Serns (1977), who studied bluegill populations in three soft-water lakes in northern Wisconsin, reported similar results; bluegills from the lake with the highest population density had the slowest growth and poorest condition. In the present study, no relation between growth and relative density of bluegills was apparent for 1-year-old fish, but as age increased, an inverse relation between population density and growth rate was increasingly evident. This observation is consistent with that of Snow (1969), who noted that bluegills from both fast- and slow-growing populations in northern Wisconsin lakes attained approximately equal length at the end of the first growing season, and with that of Backiel and Le Cren (1967), who concluded that the influence of population density on growth is most apparent for adult fish. A number of investigators have reported an inverse relation between growth rate and density of bluegill populations (Carlander 1977).

In the present study, statistical contrasts yielded similar results for both growth and condition. However, in culturally acidified waters there may be no clear-cut relation between changes in growth rate and changes in condition factor (reviewed by Harvey 1982). For example, Rosseland et al. (1980) observed increases in condition factors of larger (>20 cm) brown trout *Salmo trutta* during acidification of three lakes on the Tovdal River; growth rate of brown trout did not change, even though fish population densities (catch/unit effort) had decreased by about 90% and availability of food organisms was apparently not a limiting factor.

Menendez (1976) found that growth of brook trout alevins was significantly less at pH 5.5, 6.0, and 6.5 than at pH 7.1. Similarly, Craig and Baksi (1977) observed that growth of flagfish fry was significantly less at pH 5.0, 5.5, and 6.0 than at pH 6.8. Results of these laboratory studies suggest that it would not be unreasonable to expect bluegills to grow more slowly in the acidic lakes than in the circumneutral lakes studied here. Inasmuch as the bluegill could be qualitatively described as a fairly acid-tolerant fish species (Harvey 1980; Rahel and Magnuson 1983), perhaps a significant pH effect on growth would have been demonstrated in this study with a more acid-sensitive species. However, acid-sensitive taxa such as minnows

Table 9. Mean back-calculated total lengths (mm) of bluegills of age groups 1-4 from nine lakes[a] in north central Wisconsin. Means within a column with no letters in common are significantly different ($P \leq 0.05$; Scheffe's multiple comparison of means).

Rank of length[b]	Age (years)			
	1	2	3	4
1	84 a *Flannery*	115 a *Flannery*	139 a *Currie*	156 a Dorothy Dunn
2	81 b Garth	111 a,b Dorothy Dunn	135 a,b Dorothy Dunn	148 a,b *Currie*
3	79 b,c Dorothy Dunn	109 b *Currie*	131 b,c *Flannery*	144 b,c Clear
4	76 c *Clara*	107 b *Hilderbrand*	126 c,d Clear	143 a,c,d *Hilderbrand*
5	73 d Toulish	101 c *Clara*	125 d *Clara*	141 b,c,d *Flannery*
6	71 d,e *Hilderbrand*	98 c Clear	123 d *Hilderbrand*	140 c *Clara*
7	69 e Clear	98 c Garth	115 e Garth	134 d Toulish
8	65 f Hixon	92 d Toulish	113 e Toulish	126 e Garth
9	63 f *Currie*	80 e Hixon	95 f Hixon	105 f Hixon

[a] Names of naturally acidic lakes (pH 5.1-6.0) are italicized.

[b] Rank of 1 is maximum mean length; rank of 9 is minimum mean length.

(Cyprinidae) and darters (Percidae) were either absent or very rare in the acidic lakes. In contrast, these taxa were fairly well represented in the six circumneutral lakes, leading me to hypothesize that the chemical environments in the acidic lakes studied here were more stressful to reproduction of sensitive fish species than to growth rate inhibition of relatively acid tolerant species, such as the bluegill.

Relations between fish growth and pH are not as clear-cut in natural waters, where intrinsic (population-related) and extrinsic environmental factors affecting growth can vary simultaneously, as they are in controlled laboratory environments, where fish are often fed unrestricted rations. In the present study, extrinsic environmental variability other than that due to pH and related water chemistry characteristics was limited by studying two groups of lakes of similar morphology and hydrologic type. Changes in growth rates of fishes in surface waters undergoing cultural acidification can result from direct effects (that is, stress associated with deteriorating water quality), indirect effects from changes in intraspecific and interspecific population interactions, or a combination of direct and indirect effects (Fromm 1980; Ryan and Harvey 1977, 1980). Because acidification affects all trophic levels in aquatic ecosystems (Haines 1981), evaluation of direct stress on individual growth rates in a given species population can be difficult. In northern Wisconsin lakes, population density strongly influences condition and growth of bluegills. It is therefore concluded that growth rates and condition factors are not useful as indicators of chronic, pH-related stress on bluegill populations in natural waters.

Summary

Growth and condition of bluegills from five naturally acidic lakes (pH 5.1-6.0) and six circumneutral lakes (pH 6.7-7.5) in northern Wisconsin were compared. Although mean condition factors and mean back-calculated total lengths at ages 1 to 4 varied significantly among lakes, the differences were not related to lake pH. Rather, the ranks of mean condition factors and back-calculated lengths at ages 2, 3, and 4 were negatively correlated with relative density of bluegills among the lakes. Because of the dominating effect of population density, growth rates and condition factors are not useful as indicators of chronic, pH-related stress on bluegill populations.

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SERUM CALCIUM CONCENTRATIONS IN BLUEGILLS AND WHITE SUCKERS IN NATURALLY ACIDIC AND CIRCUMNEUTRAL LAKES

One possible mechanism for the decline of fish populations in acidified lakes is reproductive failure, which has been associated with the disruption of calcium metabolism and reproductive physiology of females in some studies. Reproductive failure in oviparous teleosts experiencing acid stress during oogenesis and oocyte development generally results from (1) failure to release mature eggs or (2) physiological changes preventing the production and development of oocytes (reviewed by Peterson et al. 1982). Under normal circumstances, mature female bony fishes have substantially elevated serum calcium concentrations prior to and during the spawning season (Fleming et al. 1964; Love 1970; Scott et al. 1980), because yolk proteins are transported from the liver to the ovary as complex calcium phosphoproteinates during development of ova. In acidified George Lake, Ontario, female white suckers exhibited depressed serum calcium concentrations, which were associated with failure to produce viable ova (Beamish et al. 1975; Lockhart and Lutz 1977). The physiological mechanisms restricting egg maturation and production in fishes experiencing acid stress appear to be the synthesis of yolk proteins and the deposition of yolk (Peterson et al. 1982). In a laboratory study of flagfish, development of oocytes to mature ova was substantially less in fish at pH 4.5, 5.0, 5.5, and 6.0 than at pH 6.7 (Ruby et al. 1977). Retardation of oogenesis at lower pHs was related to inhibition of secondary yolk deposition in the cytoplasm.

In this chapter, serum calcium concentrations in mature female fishes collected prior to active spawning in naturally acidic and circumneutral northern Wisconsin lakes are compared. Two species, bluegill and white sucker, were selected for study. The suitability of serum calcium concentrations in mature females of these species as possible indicators of chronic, pH-related stress is discussed.

Methods

Mature female white suckers were collected with fyke nets from three lakes--two acidic and one circumneutral--during early May 1981, prior to active spawning. Water temperatures were measured with a YSI model 57 dissolved oxygen meter near the area of maximum depth on each lake at 0.5 m below surface, middepth, and 0.5 m above bottom within one day of collection of fish. After collection, fish were immediately transported in large polyethylene tubs containing lake water to the University of Wisconsin, Trout Lake Biological Station near Boulder Junction, Wisconsin. Fish were anesthesized with MS-222, measured (total length) to the nearest mm, and weighed to the nearest 0.1 g. Fish were bled by heart puncture with hematocrit tubes, and serum samples were obtained by centrifugation of blood samples for 10 minutes with an International Equipment model MB micro-capillary centrifuge. Serum samples from individual white suckers were stored in Nunc Intermed cryo-tubes (32 x 12.5 mm) at -4 C until preparation for calcium analysis. Sex of each fish was determined by gonadal examination, and presence of ova in females was recorded. Female white suckers were preserved in 10% formalin for future reference.

Bluegills were collected with fyke nets from four acidic and four circumneutral lakes during mid-June 1981, prior to active spawning. Water temperatures were measured in each lake, as described above. After collection, fish were immediately transported in chilled lake water to the Trout Lake Biological Station. Fish were anesthesized, measured, sexed, and bled as described above. Due to the small volume of blood obtained from each bluegill, approximately equal volumes of serum from two to (usually) four fish were composited into a single sample for calcium analysis. Serum samples from female bluegills were obtained from all eight lakes. Samples from male bluegills were obtained from two of the eight lakes--one acidic and one circumneutral. Serum samples were collected and stored as described above. Bluegills were preserved in 10% formalin for future reference.

Determinations of calcium concentrations in serum samples were conducted at the Columbia National Fisheries Research Laboratory in Columbia, Missouri. After thawing, 75 µl of serum was mixed with 75 µl of a 10% trichloroacetic acid solution to precipitate protein from the serum sample. Two ml of a O-cresolphthalein complexone solution, followed by 2 ml of a 3.75% diethylamine solution, were added to 65 µl of the supernatant under a hood. After standing for 15 minutes, calcium concentrations were colorimetrically measured at a wavelength of 565 nm on a spectrophotometer (Baush and Lomb Spectronic 100), as described by Gitelman (1967) and Sarkar and Chauhan (1967). Serum calcium concentrations are reported as µg Ca/ml serum.

Prior to one-way analysis of variance, homogeniety of variances of serum calcium concentrations among populations was confirmed with Bartlett's test. Following one-way analysis of variance for testing lake (treatment) effects on serum calcium concentrations, paired comparisons of mean serum calcium concentrations between lakes (populations) were conducted by Tukey's honestly significant difference (*hsd*) procedure, which has an experimentwise error rate (Steel and Torrie 1960). Where sample sizes among lakes were unequal, the harmonic mean of the number of samples in the lakes was used to estimate confidence intervals for Tukey's *hsd* test (Bancroft 1968).

Results and Discussion

Serum was collected and analyzed from 64 female white suckers, all containing ova, from the three lakes (Table 10). Surface water temperatures in the three lakes were similar when these collections were made (Table 10).

Serum calcium concentrations in female white suckers differed significantly among the three lakes ($P < 0.001$; one-way analysis of variance). The mean serum calcium concentration in female white suckers from Nelson Lake (pH 7.0) was significantly greater than in those from the two naturally acidic lakes, Zottle and Currie (Table 11). Mean serum calcium concentrations in female white suckers from the two acidic lakes, both with an autumnal pH of 5.6, did not differ significantly. The mean serum calcium concentration for a sample of 16 mature female white suckers, taken from acidified George Lake in Ontario during May 1973, was lower than the mean values for white suckers from the three Wisconsin Lakes (Table 11). About 65-75% of the female white suckers in George Lake failed to release ova during 1972 and 1973 (Beamish et al. 1975). The concordant rankings of lake pH and mean serum calcium concentrations for the four lakes (Table 11) suggest that serum calcium concentration warrants further study as a possible measure of pH-related stress on female white suckers.

Waterborne calcium concentrations were not measured prior to collection of white suckers. However, the exposure concentrations of calcium in each of the three Wisconsin lakes were probably within the range of values given in Table 11. External calcium concentrations were probably higher in circumneutral Nelson Lake than in the two acidic lakes, Currie and Zottle. Waterborne calcium concentrations were also higher in acidified George Lake, Ontario (mean, 3.2 mg/l; range, 1.8-4.0 mg/l; Beamish et al. 1975), than in the two naturally acidic Wisconsin lakes; however, the mean serum calcium concentration was lower in female white suckers in George Lake than in suckers from Currie and Zottle Lakes.

Serum was collected from 215 mature female bluegills to form seven composite samples from each of six lakes and six composite samples each in two lakes (Table 12). All female bluegills analyzed contained ova. Surface water temperatures in the eight lakes were similar (range, 19.0-21.8 C) at the time of these collections (Table 12).

Serum calcium concentrations in mature female bluegills varied significantly among lakes ($P < 0.001$; one-way analysis of variance), but the differences were not related to either lake pH or waterborne calcium concentration (Table 13). Tukey's multiple comparison procedure indicated relatively broad overlap of serum calcium concentrations in female bluegills among lakes, and no consistent pattern for acidic and circumneutral lakes (Table 13). The Spearman (rank) correlation coefficient between mean serum calcium concentration and lake pH was not significant ($r_s = 0.24$). Serum calcium concentrations in female bluegills were also unrelated to waterborne calcium levels ($r_s = -0.40$). In contrast, a strong inverse relation occurred between mean serum

Table 10. Number and size of female white suckers collected for serum calcium analyses. Water temperatures in each lake during the sampling dates are also given.

Lake	Date collected (1981)	Water temperature (C)			Number of fish analyzed	Total length (mm)			Wet weight (g)		
		Surface	Middepth	Bottom		Mean	Minimum	Maximum	Mean	Minimum	Maximum
Currie	May 6	11.2	11.2	11.0	21	389	365	417	549	477	718
Zottle	May 8	12.6	8.0	9.0	24	489	456	518	1225	978	1489
Nelson	May 6-7	10.4	6.6	6.0	19	437	370	539	1100	591	2035

Table 11. Mean serum calcium concentrations in mature female white suckers from three northern Wisconsin lakes and from acidified George Lake in Ontario. Mean serum calcium concentrations (Wisconsin data) with no letters in common are significantly different ($P \leq 0.05$; Tukey's *hsd* procedure).

Lake	Location	pH[1]	Waterborne[2] calcium concentration (mg/l)	Serum calcium concentration (μg/ml)	
				Mean	CV (%)[3]
Nelson	Wisconsin	7.0	4.7/4.6	211 a	21
Zottle	Wisconsin	5.6	1.3/1.0	178 b	15
Currie	Wisconsin	5.6	2.1/1.0	168 b	21
George[4]	Ontario	4.8-5.3	1.8-4.0	144	--

[1] Autumnal pH values from Table 1.

[2] Values for Wisconsin lakes are for January 1983 (before slash) and the mean for summer (after slash; from Appendix 14).

[3] Coefficient of variation.

[4] Data from 16 fish collected during May 1973 (from Beamish et al. 1975). The pH and calcium values for George Lake water are ranges from 15 determinations made from February 1972 to August 1973.

calcium concentration and relative density of bluegills (Table 13). The Spearman correlation coefficient between relative density and mean serum calcium concentration in females (one-tailed test for negative correlation) was highly significant ($r_s = -0.83$; $P < 0.01$). Calcium concentrations in serum samples from each of the eight lakes were not linearly correlated with the mean wet weight of the bluegills from which serum was collected ($P > 0.05$ for all eight correlation coefficients).

Mean serum calcium concentrations in male bluegills from acidic Flannery Lake and circumneutral Nelson Lake (Table 13) were significantly less than in females from the same lake (t-test; $P < 0.001$ for both tests), which is normal prior to spawning (Love 1970). The ratio of mean serum calcium concentrations in females to that in males was less in acidic Flannery Lake (2.2) than in circumneutral Nelson Lake (3.1).

Analyses of serum calcium concentrations yielded contrasting results for female white suckers and bluegills. These results suggest that white suckers, which are presumably more acid-sensitive than

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Table 12. Serum calcium concentrations in bluegills from northern Wisconsin lakes. The number and size of bluegills from which each composited serum sample was obtained and water temperatures in each lake during the sampling dates are also given.

Lake category and name	Composite sample	Number and[a] sex of fish in sample	Date collected (1981)	Water temperature (C)			Total length (mm)			Wet weight (g)			Serum calcium concentration (µg/ml)
				Surface	Middepth	Bottom	Mean	Minimum	Maximum	Mean	Minimum	Maximum	
Acidic													
Clara	A	4F	June 10	19.2	17.0	10.0	156	140	185	72.6	47.8	124.5	341
	B	4F					163	151	181	87.0	59.7	125.8	305
	C	4F					150	138	160	57.5	46.5	70.3	284
	D	4F					155	148	160	58.4	53.8	68.9	321
	E	4F					157	146	165	63.5	50.9	80.1	261
	F	4F					147	141	156	52.7	44.4	60.4	219
	G	4F					150	143	154	51.9	42.9	56.5	232
Currie	A	4F	June 15	21.3	21.0	20.8	185	171	195	114.8	82.7	141.2	344
	B	4F					180	155	198	107.0	60.5	140.0	364
	C	4F					170	153	200	93.3	58.2	156.0	391
	D	4F					169	127	201	96.4	37.7	142.5	372
	E	4F					189	180	199	123.1	107.8	134.0	316
	F	4F					174	154	194	91.2	59.5	120.5	382
	G	3F					124	115	132	29.8	25.2	33.3	
Flannery	A	4F	June 12-13	20.6	19.0	15.0	138	111	154	51.3	26.5	74.8	235
	B	4F					146	123	171	61.9	36.9	94.3	376
	C	4F					165	153	189	81.2	63.4	108.5	286
	D	4F					121	110	137	33.7	21.9	53.8	253
	E	3F					155	142	169	66.5	51.8	85.8	272
	F	4F					170	161	178	96.7	89.8	104.9	305
	G	4F					154	138	166	79.9	55.6	100.7	334

Table 12. Continued.

Lake category and name	Composite sample	Number and[a] sex of fish in sample	Date collected (1981)	Water temperature (C)			Total length (mm)			Wet weight (g)			Serum calcium concentration (µg/ml)
				Surface	Middepth	Bottom	Mean	Minimum	Maximum	Mean	Minimum	Maximum	
Flannery	A	4M					146	143	150	55.8	50.7	63.2	114
	B	4M					123	122	125	33.4	31.1	37.5	140
	C	4M					136	124	147	46.1	34.5	55.8	128
	D	2M					143	135	150	49.3	39.6	59.0	92
	E	4M					141	128	161	61.1	45.4	86.2	143
	F	4M					150	124	168	70.9	38.5	97.0	139
	G	3M					122	111	129	35.7	28.8	41.7	188
Lumen	A	4F	June 15	20.8	19.0	19.2	180	152	201	86.4	48.5	135.6	262
	B	4F					171	153	195	73.1	42.9	103.5	276
	C	4F					181	161	203	88.5	56.9	128.9	228
	D	4F					166	152	185	70.0	53.3	90.0	240
	E	4F					170	160	182	69.1	47.0	87.0	250
	F	4F					172	167	176	75.9	69.0	83.9	239
	G	4F					163	155	171	63.2	53.3	70.7	220
Circumneutral													
Garth	A	4F	June 16	20.9	19.9	17.8	139	135	142	44.3	39.6	49.5	206
	B	4F					137	132	145	43.2	39.5	42.5	210
	C	4F					139	132	151	41.2	38.1	48.8	207
	D	4F					135	124	142	39.7	31.8	45.1	240
	E	4F					140	136	142	46.0	43.3	49.8	276
	F	4F					134	132	135	40.1	37.0	42.5	242
	G	4F					134	123	140	42.6	35.6	48.9	238
Hixon	A	3F	June 13	21.8	17.9	9.9	152	141	161	52.9	40.7	64.6	234
	B	3F					159	143	175	66.8	43.2	87.1	230
	C	3F					142	138	147	45.9	41.0	50.0	314
	D	3F					139	126	145	42.3	33.7	48.6	305
	E	3F					143	130	152	49.4	40.0	57.6	287
	F	3F					154	149	162	56.3	48.5	62.3	329
	G	3F					147	140	157	50.5	47.2	53.9	159

Table 12. Continued.

Lake category and name	Composite sample	Number and[a] sex of fish in sample	Date collected (1981)	Water temperature (C)			Total length (mm)			Wet weight (g)			Serum calcium concentration (μg/ml)
				Surface	Middepth	Bottom	Mean	Minimum	Maximum	Mean	Minimum	Maximum	
Nelson	A	4F	June 10-11	19.0	8.2	5.0	132	122	136	36.7	30.9	39.8	369
	B	4F					148	140	151	54.9	42.4	68.2	331
	C	4F					139	137	142	43.2	40.8	48.6	333
	D	4F					131	122	144	34.3	29.4	44.3	286
	E	4F					140	132	159	47.7	39.4	69.8	277
	F	4F					131	123	140	40.0	33.7	45.5	268
	G	4F					124	120	131	30.3	26.9	32.5	260
	A	4M					141	134	150	44.0	37.2	50.2	70
	B	4M					141	122	149	46.4	30.4	58.4	114
	C	4M					137	130	147	42.5	36.3	54.9	109
	D	4M					139	125	147	45.6	29.9	56.2	107
	E	4M					141	131	147	46.6	37.9	57.2	102
	F	4M					145	136	150	54.2	44.8	61.4	97
	G	4M					142	136	147	46.5	43.5	50.1	87
Toulish	A	4F	June 17	19.5	10.8	7.8	146	131	158	54.3	40.8	70.0	382
	B	4F					157	153	164	60.9	40.5	69.3	167
	C	4F					162	153	175	74.6	57.3	91.9	287
	D	4F					139	135	145	43.6	33.7	54.3	
	E	4F					160	140	176	69.2	43.0	96.9	319
	F	4F					165	156	171	72.7	57.5	84.8	249
	G	4F					161	154	168	64.8	54.8	74.9	325

[a] F = female; M = male.

Table 13. Mean serum calcium concentrations in mature bluegills from eight northern Wisconsin lakes. Mean calcium concentrations in a column with no letters in common are significantly different ($P \leq 0.05$; Tukey's *hsd* procedure).

Lake[1]	pH[2]	Waterborne[3] calcium concentration (mg/l)	Rank of[4] relative density	Mean serum calcium concentration (µg/ml) Females	Males
Currie	5.6	1.0	8	362 a	--
Nelson	7.0	4.6	4	303 ab	98
Flannery	5.7	1.3	7	294 ab	135
Toulish	6.7	5.7	6	288 ab	--
Clara	6.0	1.7	5	280 b	--
Hixon	6.8	4.7	3	265 b	--
Lumen	5.2	1.0	1	245 b	--
Garth	7.5	10.1	2	231 b	--

[1] Listed in order of decreasing mean serum calcium concentration; names of naturally acidic lakes are italicized.

[2] Autumnal pH values from Table 1.

[3] Mean calcium concentration during summer (from Appendix 14).

[4] Based on numbers of bluegills caught in fyke nets (Table 7); rank of 1 is highest density, and rank of 8 is lowest density.

bluegills (Harvey 1980; Rahel and Magnuson 1983), may be stressed in naturally acidic, northern Wisconsin lakes as a result of low pH, low environmental calcium, or both. In acidified George Lake, Ontario, female white suckers exhibited depressed serum calcium concentrations, which were associated with reproductive failure (Beamish et al. 1975; Lockhart and Lutz 1977).

A pH of 5.0, the minimum pH at which white suckers appeared unaffected by acid stress in the Ontario lakes surveyed by Harvey (1980), may be critical for the survival of white sucker populations. During the experimental acidification of Lake 223, Ontario, recruitment failure of white suckers occurred at pH 5.1 (Mills 1983). Laboratory exposure of larval white suckers to pH levels below 5.0 (at a calcium concentration of 2.2 mg/l) is lethal, even in the absence of toxic metals (Baker and Schofield 1982).

Direct exchange between water and the gills and skin is considered to be the major source of calcium for freshwater fishes, and the dietary contribution probably accounts for less than 10% of total calcium intake in most situations (Simkiss 1974). Yamane et al. (1982) reported that serum calcium levels in goldfish *Carassius auratus* maintained for 28 days on a calcium- and phosphorus-deficient diet (0.05% calcium) were similar to, or greater than, those in control fish fed a 0.5% calcium diet. In response to varying external calcium concentrations, fish can regulate internal calcium levels by various mechanisms. Fleming et al. (1973) found that in water free of divalent ions, the plains killifish *Fundulus zebrinus* was able to mobilize calcium from acellular bone. Shephard (1981) reported that the activity of Ca^{2+}-ATPase in the gills of roach *Rutilus rutilus* was strongly correlated with the external concentration of calcium ion and hypothesized regulation of internal calcium by the Ca^{2+}-ATPase transport system. Serum calcium concentrations in roach were independent of calcium ion concentrations in water (pH was not reported). However, the range of calcium concentrations in waters studied by Shephard (16.0-116.5 mg/l) were much higher than in the lakes studied here, and the applicability of Shephard's findings to fishes in very soft, low calcium waters is unknown. Calcium concentrations in seepage lakes of northern Wisconsin may be universally low. Juday et al. (1938) found that 85% of 128 seepage lakes in the Highland Lake District (defined primarily by the study area in this investigation; Fig. 1) of northern Wisconsin had calcium concentrations less than 2.0 mg/l; the maximum calcium concentration measured in a seepage lake was 6.1 mg/l.

In temperate waters, temperature and photoperiod are considered to be the environmental factors that control the annual reproductive cycle of fishes (Billard and Breton 1978). White sucker populations have synchronous oocyte development with distinct and brief annual breeding cycles. Peak spawning of a white sucker population is apparently triggered by increasing water temperature and may occur within a 48-hour period (Corbett and Powles 1983). In contrast, the reproductive cycle of bluegill populations is asynchronous, and spawning can continue from late spring until mid-summer (Scott and Crossman 1973). Variation in the initiation and duration of the reproductive cycle of bluegill populations can occur among lakes with differing water temperatures. However, mean serum calcium concentrations in female bluegills were not correlated with water temperatures at 0.5 m below surface (r_s = -0.29) or middepth (r_s = -0.14) in the eight lakes studied here. The strong negative correlation observed between mean serum calcium concentration in female bluegills and relative density of bluegill populations suggests that some density-dependent factor, such as nutritional status, may also affect the reproductive cycle in this species. Both condition and growth rates of bluegills were inversely related to population density in these lakes (see preceding chapter).

The potential utility of serum calcium concentrations measured prior to spawning as an indicator of acid stress is probably much greater for synchronous spawners, such as the white sucker, than for asynchronous spawners, such as the bluegill. Because the timing of spawning can vary among lakes, a time series of measurements of serum calcium during the reproductive cycle would be more useful than measurements made at a single point in time. The potential of this measure as a biological monitoring tool to detect chronic acid stress in fish populations with synchronous reproductive cycles merits further study. The data presented in Table 11 offer one working hypothesis--that serum calcium concentration prior to spawning is depressed in mature female white suckers exposed to pH 5.6-5.8 or lower at waterborne calcium concentrations less than 2.0 mg/l.

Summary

Serum samples were collected from mature white suckers and bluegills from selected Wisconsin lakes during spring 1981, prior to spawning. Female white suckers were collected from a lake with neutral pH (7.0) and from two naturally acidic lakes (pH 5.6). The mean serum calcium concentration in female white suckers from the neutral lake was significantly greater than that in those from the acidic lakes, whereas mean concentrations in female white suckers from the two acidic lakes did not differ. Serum calcium concentrations in female bluegills from four acidic and four circumneutral lakes varied among lakes; however, no relation between serum calcium concentration and either lake pH or waterborne calcium was apparent. In contrast, a strong negative correlation between mean serum calcium concentration and relative density of bluegills in the eight lakes was observed, suggesting that the timing of the reproductive cycle of bluegills is related to nutritional status. Mean serum calcium concentrations in mature male bluegills, measured in samples from one acidic and one circumneutral lake, were substantially less than in female bluegills from the two lakes, which is the norm prior to spawning. It was hypothesized that white suckers, which are presumably more acid-sensitive than bluegills, are experiencing chronic pH-related stress in naturally acidic Wisconsin lakes with annual pH ranges of 5.6-5.8 or less and with waterborne calcium concentrations less than 2.0 mg/l. The utility of serum calcium concentrations, measured prior to spawning, as a measure of pH-related stress on populations of white suckers and other synchronous spawners merits further study. This measure is probably not useful for evaluating acid stress on fish species that have asynchronous reproductive cycles.

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TRACE METAL ACCUMULATION BY BLUEGILLS AND WALLEYES: RELATION TO LAKE pH

Acidification of surface waters can accelerate rates of accumulation of certain toxic metals by fish. Uptake of both organic and inorganic Hg by fish increases with increasing hydrogen ion concentration; consequently, piscivorous fishes from naturally acidic and culturally acidified surface waters often contain elevated concentrations of Hg (reviewed by Haines 1981). Acidic precipitation can also accelerate the fluxes of other toxic metals such as Al, Cd, Cu, Mn, Pb, and Zn from the edaphic to the aquatic environment (Wright and Gjessing 1976; Forstner 1982; Nriagu et al. 1982; Schindler and Turner 1982). Increased accumulation of Pb with decreasing pH has been demonstrated with fish at chronic exposure concentrations in laboratory studies (Merlini and Pozzi 1977a; Hodson et al. 1978b). However, effects of pH and related chemical characteristics on biological availability and accumulation of potentially toxic metals other than Hg have been studied little in lakes.

In this chapter, the relation between concentrations of selected metals in fishes and the pH of northern Wisconsin lakes is evaluated. Whole bluegill was selected as the matrix to be analyzed for Al, Cd, Cu, Pb, Mn, V, and Zn, because bluegills are generally abundant in northern Wisconsin lakes and are good indicators of surface water contamination by certain metals (Atchison et al. 1977; Murphy et al. 1978). In addition, reliable background information is available on trace metal concentrations in whole bluegills from metal-contaminated and relatively uncontaminated surface waters in the United States.

Mercury concentrations are generally highest in piscivorous fishes, such as walleye and northern pike (Scott 1974; Phillips et al. 1980). Muscle tissue from adult walleyes, which are largely piscivorous (Ney 1978; Ryder and Kerr 1978), was consequently selected as the matrix to be analyzed for Hg.

Methods

Bluegills and walleyes were collected with 91.4-m, variable mesh gill nets and small mesh (0.95-cm) fyke nets during the summer 1980 fishery survey. After collection, fish were placed into plastic bags and held in ice-filled chests at the sampling site. Samples were frozen within 12 hours after capture and stored at about -4 C until measurement. Each fish was weighed to the nearest 0.1 g and measured (total length) to the nearest millimeter. Scales for age determinations were taken from each fish immediately posterior to the point of insertion of the left pectoral fin. Scale impressions were made and examined at a magnification of 38X on an Eberbach scale reader. Age assigned to each fish was equal to the total number of completed scale annuli, according to criteria described by Tesch (1971). Precautions were taken to prevent contamination of fish samples during handling and storage.

Preparation and Digestion of Fish Samples

Analyses of Al, Cd, Cu, Mn, Pb, V, and Zn were conducted on two composite samples of bluegills per lake from five acidic and five circumneutral lakes (Table 14). Each composite sample contained from three to (usually) five whole bluegills of a single age class. All bluegill samples analyzed were composed of four-year-old fish, with the exception of the composite samples from Lumen Lake, which contained six- and seven-year-old fish (Table 14).

Sample homogenizations, digestions, and analyses were conducted at the Columbia National Fisheries Research Laboratory in Columbia, Missouri. Whole bluegills comprising each composite sample were initially homogenized with a Hobart model 4612 meat grinder. One 125-g aliquant was taken from each initial homogenate of whole bluegills. For further homogenization, tissues were placed with deionized water in a 200-ml glass vertical flute flask and blended at 10,000 rpm with a Tekmar SD-45 homogenizer equipped with a G-450 generator and Teflon bearing shaft (SD-45N). All sample-water mixtures were prefrozen to -30 C in an FTS Systems freeze dryer. Lyophilization was initiated with a condenser coil temperature of -65 C and vacuum at <200 mToor. A vacuum level control monitored vacuum at approximately 300 mToor throughout the lyophilization cycle to improve system thermal conductivity. Sample, shelf, and condenser coil temperatures and vacuum were monitored with a Leeds and Northrup Model 250 recorder. All samples were lyophilized with shelf temperatures approximating the lowest eutectic point for whole ground fish (--4 C). Following lyophilization, two 0.5-g (dry weight) aliquants were taken from each homogenate for replicate analysis.

Samples of axial (edible) muscle for Hg analysis were taken from a total of 40 walleyes from two acidic and three circumneutral lakes. Only four-, five-, and seven-year-old walleyes were analyzed. Axial muscle samples were dissected from the area lateral and ventral to the dorsal fin of each fish with stainless steel implements on an acid-washed, polyethylene work surface. Polyethylene gloves were worn during dissections. Axial muscle samples were placed in acid-washed polyethylene vials and stored at -4 C until preparation for analysis.

Samples of whole bluegill (0.5-g dry weight aliquants) and walleye muscle tissue (2.0-g wet weight) were digested overnight in Lab Glass pressure reaction bottles (42-ml size) containing concentrated

Table 14. Description of composite samples of whole bluegills analyzed for Al, Cd, Cu, Mn, Pb, V, and Zn.

Lake category and name	Composite sample	N	Age (years)	Total length (mm)			Wet weight (g)		
				Mean	Minimum	Maximum	Mean	Minimum	Maximum
Acidic									
Clara	1	5	4	141	131	155	45.7	36.1	58.2
	2	5	4	139	129	164	43.6	31.6	68.7
Currie	1	5	4	171	164	181	89.9	75.7	119.8
	2	5	4	166	159	173	86.1	78.5	95.9
Flannery	1	5	4	137	122	165	48.6	34.5	81.0
	2	5	4	133	125	141	41.0	33.1	48.8
Hilderbrand	1	5	4	136	129	141	42.5	37.4	48.1
	2	5	4	136	121	150	44.6	26.9	61.0
Lumen	1	5	6	168	164	171	65.9	57.8	74.1
	2	5	7	179	166	212	88.7	66.3	160.0
Circumneutral									
Clear	1	5	4	140	117	153	54.3	28.3	66.7
	2	5	4	134	125	143	41.7	31.8	52.0
Garth	1	3	4	134	125	147	36.5	30.2	48.2
	2	3	4	116	107	131	23.6	15.7	32.0
Hixon	1	5	4	115	108	129	26.2	19.3	37.5
	2	5	4	106	101	110	19.2	15.7	23.4
Nelson	1	4	4	114	102	130	21.3	14.9	30.7
	2	4	4	125	99	151	31.3	14.1	49.7
Toulish	1	5	4	142	126	156	49.7	31.9	64.7
	2	5	4	133	126	137	33.7	27.8	39.1

sub-boiled HNO_3. The vessels were capped with a Teflon-lined, polyethylene sheet-crown cap sealing assembly and placed in aluminum blocks submerged in a water bath, which was slowly brought from room temperature to 65 C and maintained there for 48 hours. Digestates were quantitatively transferred and diluted to a final volume of 50 ml with 1% HCl. Diluted digestates of bluegill samples were stored in preleached (Karin et al. 1975) conventional polyethylene bottles (Moody and Lindstrom 1977) prior to analysis. Diluted digestates of walleye muscle tissue were stored in borosilicate test tubes prior to Hg analysis; hydrochloric acid was routinely added to these digestates to prevent low level Hg losses during storage (Koirtyohann and Khalil 1976). All HNO_3 and HCl used for preparing standards and samples were Baker Reagent Grade and were distilled in sub-boiling quartz evaporators (type PB 5, Quartz Products Corporation) prior to use.

Analyses of Fish Samples

Concentrations of Al, Cd, Cu, Mn, Pb, and V were determined by nonflame atomic absorption spectrophotometry with a Perkin-Elmer model 5000 spectrophotometer equipped with an HGA-500 graphite furnace and an AS-1 autosampling system. A four-point standard additions procedure was performed on each diluted digestate to correct for matrix interferences, as described by May and McKinney (1981) and Wiener et al. (in press). Absorbance values were printed on a Superterm terminal (Intertec Data Systems), with final values being the average of duplicate 10-µl injections. If the correlation coefficient (*r*) for the regression line was below 0.999, the data were rejected, and corresponding digestates were rerun. Concentrations of Zn were determined by flame atomic absorption spectrophotometry. All analytical standards were prepared from Fisher reference solutions.

Concentrations of Hg were determined by the flameless or cold vapor atomic absorption technique adapted from that of Koirtyohann and Khalil (1976). A Perkin-Elmer Model 5000 spectrophotometer was equipped with a Pyrex tube absorption cell (100-mm long and 6-mm i.d.) with quartz end windows and Pyrex side arms (4-mm o.d.) for vapor passage. The cell was heated with a Cole-Parmer Dyna Lume model 3151-6 high intensity radiant heat projector, to 35-40 C to prevent condensation on the end windows. The analytical system was automated with a Technicon Autosampler IV and Proportioning Pump III with appropriate pump tubes, pulse suppressors, mixing coils, and phase separator (May and McKinney 1981). Peak heights were measured from recorder chart tracings, and Hg concentrations were calculated from the standard line.

Quality Assurance Procedures

Sample preparation and analytical procedures were evaluated by (1) analysis of appropriate U.S. National Bureau of Standards (NBS) reference materials (bovine liver, tuna, oyster tissue, and water), (2) determination of percent recovery from spiked fish samples, and (3) analysis of replicate samples.

Results and Discussion

Quality Assurance

Analyses of NBS standard reference materials for Cd, Cu, Hg, Mn, Pb, V, and Zn indicated that accuracy was almost always within 10% of the certified concentration range (Table 15). Unfortunately, the NBS standard reference materials analyzed were not certified for Al. Mean recovery of the eight metals (including Al) from spiked samples ranged from 95.3 to 111% (Table 16). Precision of Hg analyses (relative standard deviation), calculated from duplicate analyses of five walleye muscle samples, ranged from 0 to 7.8%.

The following approach was used to measure the similarity of metal concentrations between replicate 0.5-g (dry weight) aliquants taken from each homogenized composite of whole bluegills. Each replicate from a composite sample was randomly assigned a value of 1 or 2. For each metal analyzed, the concentration in replicate 1 was regressed against that in replicate 2 ($n = 20$ for each simple linear regression). The coefficients of determination (r^2) of the regressions were: Al, 0.85; Cd, 0.97; Cu, 0.997; Mn, 0.98; Pb, 0.99; V, 0.78; Zn, 0.96. The high correlations of metal concentrations between replicate samples from composites validate our homogenization and subsampling procedures for composites of whole bluegill. Furthermore, excellent precision is indicated for metal analyses of whole bluegills.

Metal Concentrations in Whole Bluegill

The mean concentrations of metals in individual composite samples, calculated from replicate subsamples, were used in further analyses. Mean concentrations of Al and Cu differed substantially between the two composite samples from certain lakes (Table 17); this may have resulted from variation of Al and Cu concentrations in whole bluegills or from contamination of certain composite samples during homogenization, given that Al and Cu concentrations in replicate subsamples of individual composites agreed closely.

Concentrations of Cd, Mn, Pb, V, and Zn in whole bluegills varied significantly ($P < 0.01$) among lakes, whereas concentrations of Al and Cu did not (one-way analysis of variance; $n = 2$ for each lake). The overall mean concentrations of Cd and Pb in whole bluegills from the ten lakes (the average of the two values given for each metal and lake in Table 17) increased with decreasing lake pH (Fig. 4). Spearman correlation coefficients (r_s) between lake pH and mean Cd and Pb concentrations in bluegills were -0.867 and -0.864, respectively ($P < 0.01$ for both r_s). The pH-related increases in Cd and Pb concentrations in whole bluegills were evident only in the pH $\leq$ 6.0 range (Fig. 4). Concentrations of the two metals in bluegills varied little among the five circumneutral lakes. Cadmium and Pb levels in bluegills were also inversely correlated with mean waterborne calcium

Table 15. Results of analyses of National Bureau of Standards (NBS) reference materials for trace metals. Values for bovine liver, oyster tissue, and tuna are µg/g dry weight. Values for water are µg/g.

Element	NBS reference material	NBS certified concentration range	Our results		
			n	Concentration (X ± 1SD)	Percent recovery (range)
Cd	Bovine liver	0.23-0.31	3	0.29 ± 0.01	100[a]
	Oyster tissue	3.1-3.9	2	3.44 ± 0.07	100
	Water	0.009-0.011	2	0.010 ± 0.000	100
Cu	Bovine liver	183-203	3	186 ± 10	97-100
	Oyster tissue	59.5-66.5	2	61.2 ± 3.1	99-100
	Water	0.016-0.020	2	0.017 ± 0.000	100
Hg	Bovine liver	0.014-0.018	2	0.0195 ± 0.0035	100-122
	Oyster tissue	0.042-0.072	1	0.042	100
	Tuna	0.85-1.05	2	0.91 ± 0.01	100
	Water	0.0010-0.0012	1	0.0011	100
Mn	Bovine liver	9.3-11.3	3	10.6 ± 1.1	100-105
	Oyster tissue	16.3-18.7	2	19.4 ± 0.6	101-106
Pb	Bovine liver	0.26-0.42	3	0.39 ± 0.04	100
	Oyster tissue	0.44-0.52	2	0.39 ± 0.01	88-90
	Tuna	0.46[b]	2	0.55 ± 0.01	--
	Water	0.026-0.028	2	0.027 ± 0.001	100
V	Oyster tissue	2.8[b]	4	2.1 ± 0.2	--
	Water	0.050-0.056	5	0.055 ± 0.007	90-110
Zn	Bovine liver	117-143	3	135 ± 4	100
	Oyster tissue	838-866	2	832 ± 2	99

[a] A single value of 100 indicates 100% recovery for all replicates.
[b] Concentration not certified.

Table 16. Recovery of trace metals from spiked samples.

Element	Mean recovery (%)	Standard deviation (%)
Al	108	11.6
Cd	104	17.0
Cu	96.7	9.3
Hg	100	4.2
Mn	111	14.8
Pb	95.3	3.9
V	109	11.2
Zn	102	5.3

concentrations (Table 1) in these lakes (r_s = -0.92 and -0.87, respectively; $P < 0.01$ for both r_s, one-tailed tests for negative correlation). However, variation in Pb concentrations in bluegills among the acidic lakes was more strongly related to pH than to waterborne calcium (Fig. 4 and Table 1).

The mean and maximum Cd and Pb concentrations in whole bluegills from the acidic Wisconsin lakes were similar to or exceeded values reported for whole bluegills from U.S. lakes and rivers contaminated by metal-containing industrial effluents and urban runoff (Table 18). In contrast, Cd and Pb concentrations in bluegills from the circumneutral Wisconsin lakes were similar to values reported for bluegills from uncontaminated waters (Table 18). Given the remote location of the Wisconsin lakes, the close proximity and geological similarity of the watersheds of the acidic and circumneutral lakes, and the absence of direct anthropogenic inputs of metals to the lakes, these findings suggest that Cd and Pb in the naturally acidic lakes are highly available to fish and perhaps other aquatic biota.

Increased Pb accumulation by fish with decreasing pH has been observed in laboratory studies. Working with juvenile rainbow trout, Hodson et al. (1978b) found that the equilibrium Pb concentration in blood increased by a factor of 2.1 for each unit decrease in pH. Merlini and Pozzi (1977a) found that pumpkinseed exposed to Pb accumulated about three times more Pb at pH 6.0 than at pH 7.5.

Fish readily accumulate free Cd^{2+} and Pb^{2+}, and direct uptake from water is apparently the primary mode of uptake of these metals by fish (Merlini and Pozzi 1977b; Hodson et al. 1978a; Williams and Giesy 1978; Kumada et al. 1980; Part and Svanberg 1981). As hydrogen ion concentration increases, the availability of Cd and Pb to fish is increased by a number of possible mechanisms. With decreasing pH in the pH range studied here (5.1-7.5), proportionately more Cd and Pb should exist as the free divalent ion (Davies et al. 1976; Griffin and Shimp

Table 17. Average metal concentrations in dual composite samples of whole bluegills from ten northern Wisconsin lakes. Lakes are listed in order of increasing pH.

Lake	pH[a]	Composite sample	Mean concentration (µg/g dry weight) Al	Cd	Cu	Mn	Pb	V	Zn
Hilderbrand	5.1	1	5.7	0.465	3.13	84.4	3.92	0.140	105
		2	3.8	0.470	3.25	52.4	3.86	0.110	107
Lumen	5.2	1	16.1	0.560	1.66	61.5	3.46	0.330	148
		2	17.7	0.650	3.24	61.7	4.04	0.385	135
Currie	5.6	1	13.9	0.080	2.37	57.5	1.70	0.240	98
		2	6.9	0.085	1.81	62.1	1.50	0.310	104
Flannery	5.7	1	11.8	0.485	5.04	41.2	1.21	0.105	141
		2	6.9	0.480	3.48	30.2	1.16	0.100	132
Clara	6.0	1	4.8	0.075	1.67	26.9	0.44	0.170	115
		2	11.2	0.080	2.21	31.3	0.64	0.215	101
Toulish	6.7	1	9.4	0.045	4.30	31.8	0.22	0.195	91
		2	10.0	0.055	2.80	31.1	0.30	0.150	100
Hixon	6.8	1	24.6	0.045	5.27	69.2	0.25	0.135	135
		2	15.4	0.035	5.36	108.7	0.20	0.255	127
Clear	6.9	1	6.2	0.035	3.41	29.9	0.26	0.120	102
		2	4.9	0.035	3.21	36.1	0.26	0.090	108
Nelson	7.0	1	25.0	0.075	6.21	49.0	0.35	0.170	108
		2	8.2	0.055	7.68	50.1	0.25	0.160	115
Garth	7.5	1	4.4	0.020	2.38	28.3	0.19	0.055	110
		2	6.7	0.025	7.55	45.1	0.31	0.090	113

[a] Autumnal pH values from Table 1.

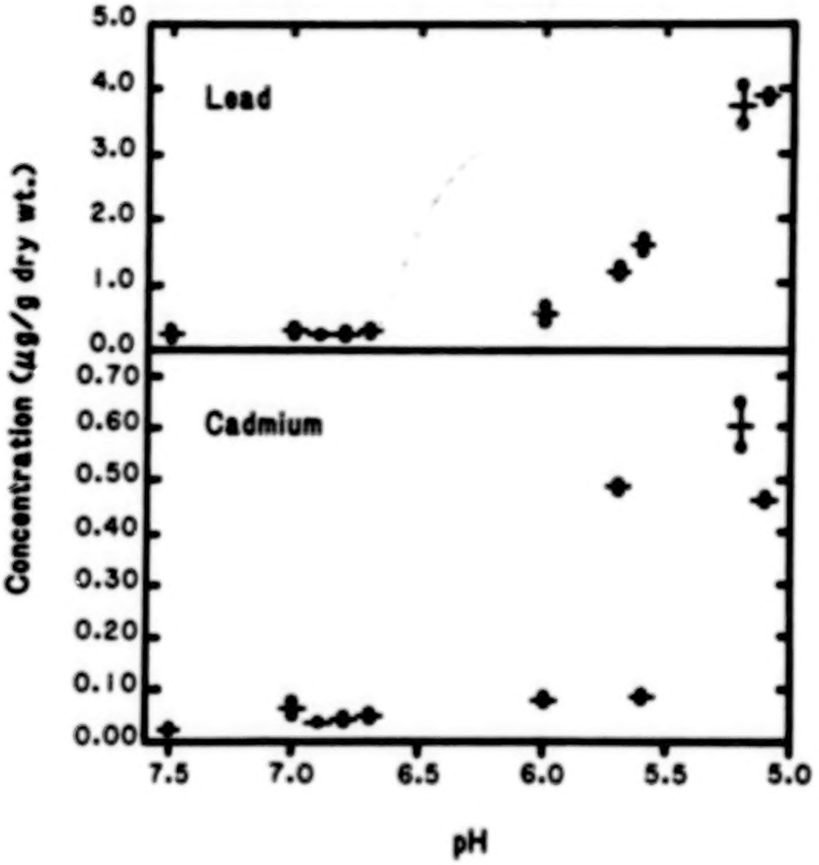

Figure 4. Cadmium and lead concentrations in whole bluegills in relation to pH of ten lakes in north central Wisconsin. Each point is the mean concentration in a composite sample of whole bluegills. Horizontal bars are the means of the two composite samples from each lake.

1976; Stumm and Morgan 1981), the form of these metals most readily assimilated through the gills of fish (Merlini and Pozzi 1977b; Part and Svanberg 1981). Solubility of Cd and Pb also increases with decreasing pH in the pH range studied here (Stumm and Morgan 1981). Lastly, the permeability of fish gill membranes increases with increasing hydrogen ion concentration (Haines and Schofield 1980; McDonald 1983). The direct uptake of divalent metals by fish also appears to be affected by waterborne calcium. The toxicity to fish of divalent metals such as Cd and Pb decreases as concentrations of the hardness ions, calcium and magnesium, increase. Pagenkopf (1983) attributed this effect to competition between the toxic divalent metals and the hardness ions for gill surface interaction sites. McFarlane and Franzin (1980), who studied variation in metal accumulation by fishes among five Canadian lakes (pH range, 7.8-8.4), hypothesized that waterborne calcium reduced the rate of accumulation of Cd and Cu by fish. Additional research is being conducted in the lakes studied here to evaluate the relative effects of waterborne calcium, pH, and waterborne metal concentrations on the accumulation of Cd and Pb by resident fishes.

Although mean concentrations of Mn, V, and Zn in whole bluegills varied among the ten study lakes, they were unrelated to lake pH and did

Table 18. Mean cadmium and lead concentrations in whole bluegills from acidic and circumneutral northern Wisconsin lakes, relative to the range of mean concentrations in whole bluegills from other surface waters in the United States. Concentration ranges for bluegills from individual northern Wisconsin lakes are indicated in parentheses.

	Mean concentration (µg/g dry weight)		
Location	Cadmium	Lead	Reference
Acidic Wisconsin lakes	0.34 (0.08-0.61)	2.19 (0.54-3.89)	This study
Metal-contaminated lakes	0.36-3.4	6.1	Atchison et al. (1977)
Upper Mississippi River	0.01-0.11	0.16-1.09	Wiener et al. (in press)
Circumneutral Wisconsin lakes	0.04 (0.02-0.07)	0.26 (0.23-0.30)	This study
Uncontaminated lakes	0.04-0.09	0.47	Atchison et al. (1977) Wiener and Giesy (1979)

not exhibit the many-fold (~20X) variation among lakes shown by Cd and Pb. Manganese, V, and Zn are known essential trace elements, whereas Cd and Pb are not (Mertz 1981). Internal concentrations of Mn, Zn, and other essential elements appear to be homeostatically controlled and are consequently less variable than those of Cd and Pb (Giesy and Wiener 1977; Wiener and Giesy 1979). The accumulation of waterborne V by fish is slow (Bell et al. 1981), and bioconcentration factors for this metal are low in fish (Holdway et al. 1983). Fish apparently regulate internal V concentrations by varying the efficiency of uptake, rate of elimination, or both (Stendahl and Sprague 1982; Holdway et al. 1983). Unlike Cd and Pb, species of V in water do not complex with carbonates and bicarbonates, and the acute toxicity of waterborne V to fish is not greatly affected by pH or water hardness (Stendahl and Sprague 1982).

The extent to which Al is accumulated by fish has been little studied. It is, however, known that the mode of Al toxicity to fish is external (on the gills), not internal (Muniz and Leivestad 1980). Buergel and Soltero (1983) studied the effect of alum (aluminum sulfate) addition to a eutrophic Washington lake (pH ~9) on Al concentrations in several tissues of stocked rainbow trout. Although total and dissolved (<0.45 µ) Al concentrations in water were far greater in the alum-treated lake than in nearby, untreated lakes, no clear pattern of increased Al accumulation by rainbow trout from the alum-treated lake was evident.

Information on metal concentrations in lake sediments was not collected during this study, but the surficial sediments of lakes in north central Wisconsin are known to be contaminated with Pb from automobile emissions (Iskandar and Keeney 1974). However, it is unlikely that desorption of Pb from sediments occurs in the pH range of the lakes studied here (Davis et al. 1982; Forstner 1982), except under anoxic conditions. The surficial sediments of northern Wisconsin lakes also appear to be enriched with Cd, but to a lesser extent than that observed for Pb (Iskandar and Keeney 1974).

Existing data on the Cd and Pb content of precipitation in north central Wisconsin are scanty. Concentrations of Cd and Pb in bulk precipitation (presumably snow), collected in Vilas County from late November 1980 until early March 1981 by the U.S. Geological Survey (Peters and Bonelli 1982), were typical of values reported for rural areas (cf. Galloway et al. 1982b). Lead concentrations in bulk precipitation samples from Vilas County ranged from <1 to 8 μg/l, whereas Cd concentrations were <1 μg/l in all samples (n = 8; Peters and Bonelli 1982). In view of the results of the present study, detailed information on atmospheric metal inputs to lakes in north central Wisconsin would be useful.

Mercury Concentrations in Walleye Muscle Tissue

Raw data on total Hg concentrations in individual walleye are given in Appendix 20. Mercury concentrations in axial muscle tissue were greater in walleyes from the two naturally acidic lakes than in fish from the three circumneutral lakes (Fig. 5 and Table 19). One-tailed Mann-Whitney tests revealed that Hg concentrations were significantly greater in four- ($P < 0.001$), five- ($P < 0.001$), and seven-year-old walleyes ($P < 0.01$) from acidic lakes (data from Currie and Clara Lakes combined) than in fish of the same age from the circumneutral lakes (data from Clear, Dorothy Dunn, and Nelson Lakes combined). Scheider et al. (1979) compared Hg concentrations in walleyes from 52 Ontario lakes and reported similar results; fish from the low alkalinity lakes (<15 mg/l as $CaCO_3$) contained more Hg than fish of the same size from high alkalinity lakes (>15 mg/l as $CaCO_3$). Suns et al. (1980) found that total Hg concentrations in whole, yearling yellow perch in 14 Ontario lakes (pH range, 5.1-7.5) were inversely correlated with epilimnetic pH, but were not correlated with alkalinity. Glazer and Bohlander (1978) reported total Hg concentrations in axial muscle tissue ranging from 0.03-2.67 μg/g in individual walleyes from 11 lakes in northeastern Minnesota, similar to the range of values observed here (Appendix 20). Mercury contamination of walleyes varied substantially among the 11 Minnesota lakes, but no attempt was made to relate Hg concentrations in fish to chemical factors.

Muscle samples from 8 of the 22 fish analyzed from the two acidic lakes contained total Hg concentrations in excess of the current U.S. Food and Drug Administration (FDA) 1.0 μg/g (wet weight) "Action Level", which has been in effect since June 1978. Twenty of the 22 fish contained Hg concentrations greater than or equal to 0.5 μg/g wet

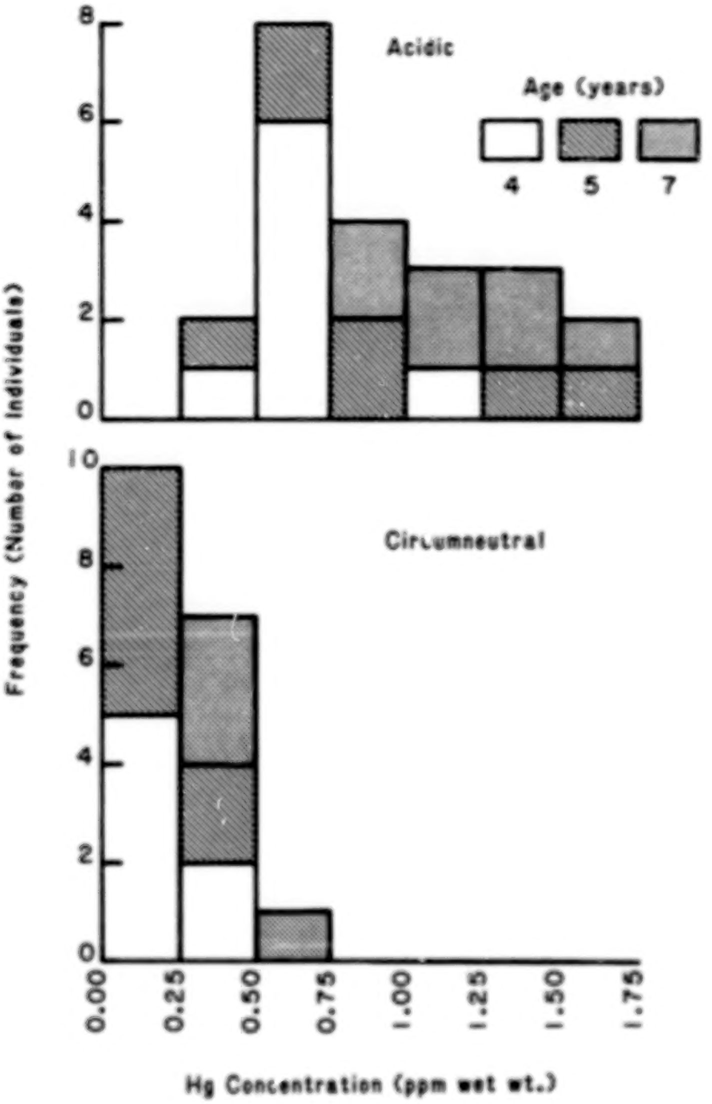

Figure 5. Frequency histograms of mercury concentrations in axial muscle tissue of walleyes from acidic (Currie and Clara) and circumneutral (Clear, Dorothy Dunn, and Nelson) lakes in north central Wisconsin.

weight. None of the 18 walleyes from the three circumneutral lakes contained Hg concentrations in excess of the current FDA action level. Although methylmercury was not measured in these samples, it is likely that 80% or more of the Hg in these fish was in the highly toxic, monomethylated form (Huckabee et al. 1979). Mercury concentrations in walleyes from the three circumneutral lakes were near values considered to be natural background levels by some authors (see review by Huckabee et al. 1979). Kelly et al. (1975) reported Hg concentrations of about 0.2 ± 0.05 µg/g (wet weight) in muscle tissue of 28-cm walleyes from Houghton Lake (Michigan), which is relatively uncontaminated.

Mercury concentrations in walleyes from the two naturally acidic lakes (data combined) increased concomitantly with body size (Fig. 6)

Table 19. Mean total mercury concentrations (µg/g wet weight) in axial muscle tissue of walleyes from northern Wisconsin lakes, by age. Ranges are given in parentheses with means. Lakes are listed in order of increasing pH.

Lake	pH	Age (years)		
		4	5	7
Currie	5.6	0.77 (0.50-1.10) n=3	0.87 (0.41-1.41) n=3	1.11 (0.82-1.43) n=4
Clara	6.0	0.57 (0.49-0.68) n=5	0.92 (0.59-1.65) n=4	1.34 (0.89-1.74) n=3
Clear	6.9	0.15 (0.12-0.17) n=2	0.22 (0.18-0.24) n=5	--
Dorothy Dunn	6.9	0.24 (0.22-0.27) n=5	0.31 -- n=1	0.34 (0.32-0.35) n=2
Nelson	7.0	--	0.38 -- n=1	0.47 (0.36-0.57) n=2

and were positively correlated with wet weight ($r = 0.440$, $P < 0.05$), total length ($r = 0.636$, $P < 0.01$), and age ($r = 0.589$, $P < 0.01$). In fish from circumneutral lakes, Hg concentrations were significantly correlated with age ($r = 0.714$, $P < 0.01$), but not with weight or total length (both $r < 0.20$). Because of their limited predictive potential (r^2 for significant r ranged from about 0.2 to 0.5), linear regression equations are not presented for these relationships. Several investigators have reported positive correlations between size or age of fish and Hg concentration (Huckabee et al. 1979).

Historical information on the Hg content of fish from these lakes are not available. However, atmospheric inputs of Hg to lakes in this area have probably increased in recent decades. Syers et al. (1973) reported enrichment of Hg in surficial sediments in three lakes near the lakes studied here. One of their study lakes (Crystal Lake) was an oligotrophic seepage lake similar to the lakes in this study. Mercury concentrations in Crystal Lake were about four-fold greater in surficial sediments than in deeper, precultural sediments. Syers et al. (1973) hypothesized that atmospheric inputs accounted for the increased Hg concentrations in more recent sediments of northern Wisconsin lakes. Working with walleyes from Michigan Lakes, Kelly et al. (1975) compared Hg concentrations in muscle tissue from museum (1865-1936) and recent (1971) samples and found significant ($P < 0.05$) temporal increases in Hg concentrations in walleyes from three of seven lakes studied. The increases in mean Hg concentrations (adjusted for standard length or

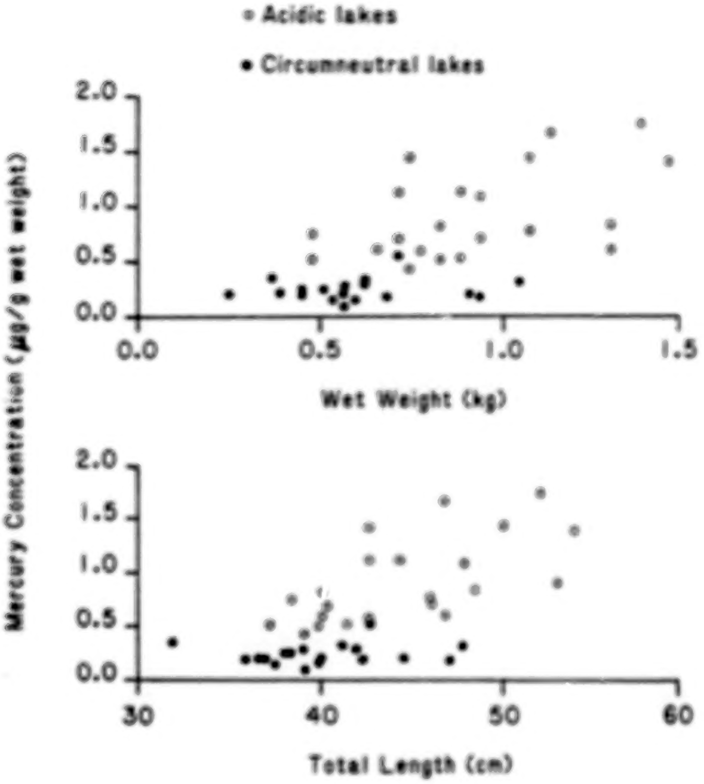

Figure 6. Plots of mercury concentrations in walleyes from acidic (open circles) and circumneutral (closed circles) lakes in north central Wisconsin against wet weight and total length.

age) in recent versus museum specimens in the three lakes ranged from 27 to 73%.

The effects of pH and related chemical factors on Hg uptake by fish have been recently reviewed (Akielaszek and Haines 1981; Haines 1981). Mercury may be more biologically available in acidic than in circumneutral lakes as a result of increased methylation rates, solubility in water, gill membrane permeability, or a combination of these and other factors. Tomlinson et al. (1980) suggested that Hg concentrations may be elevated in fishes in acidic waters because of reduced growth rates (i.e., greater Hg availability per unit of biomass). However, growth of walleyes was apparently faster in the two acidic lakes than in the three circumneutral lakes studied here (Fig. 6).

The majority of Hg in fish, usually more than 80%, is in the methylated form (Huckabee et al. 1979). Fish cannot methylate inorganic Hg in vivo (Huckabee et al. 1978), and methylmercury is accumulated by fish as a result of uptake from both food and water, coupled with a very slow rate of elimination (Phillips and Buhler 1978; Huckabee et al. 1979). The relative importance of dietary assimilation and direct uptake from water of Hg by walleyes is not known. Walleyes fed

Hg-contaminated fish do accumulate Hg in axial muscle and certain other tissues (Scherer et al. 1975), but methylmercury in forage fish ingested by piscivorous fishes is probably less readily assimilated than previously thought (Phillips and Gregory 1979). Rodgers and Qadri (1982) estimated that yearling yellow perch in the Ottawa River, Ontario, obtained 62% of their body burdens of organic Hg from food and 38% from water.

The direct uptake of waterborne methylmercury by fish occurs almost entirely across the gills (Olson et al. 1973). The *efficiency* of this direct uptake is apparently enhanced in waters with low pH and low calcium concentration, because of increased gill membrane permeability under these conditions (Rodgers and Beamish 1983; McDonald 1983). However, increased dietary uptake could also occur under these conditions due to increased Hg accumulation by organisms in lower trophic levels. For example, Stokes et al. (1983) found that Hg accumulation by filamentous green algae grown on artificial substrates was greater in four Precambrian Shield lakes with pH ranging from 5.4 to 5.9 than in a neutral lake (pH 7.2). Most of the Hg in these algae occurred in the methylated form, and total Hg concentrations in algae and fish from the five lakes (yearling yellow perch analyzed by Suns et al. 1980) were linearly correlated ($r = 0.93$). MacCrimmon et al. (1983) compared total Hg concentrations in axial muscle tissue of lake trout *Salvelinus namaycush* from nine neighboring Ontario lakes and observed substantial differences in the Hg concentration-body size relationship among populations (lakes). Standardized (to a common length) Hg concentrations in lake trout from the nine lakes were strongly correlated ($r = 0.96$) with standardized concentrations of Hg in rainbow smelt *Osmerus mordax*, a principal forage fish.

In view of the potential health effects of consuming Hg-contaminated fish, additional study of Hg in fishes of acidic lakes in this area is warranted. An additional survey of Hg contamination, which includes more lakes and fish species than studied here, has been undertaken by the Wisconsin Department of Natural Resources. Other piscivorous species, such as largemouth bass, smallmouth bass, northern pike, muskellunge, sauger, and lake trout, are known to accumulate substantial quantities of Hg in edible muscle tissue (Abernathy and Cumbie 1977; Bloomfield et al. 1980; Phillips et al. 1980; Akielaszek and Haines 1981; Wren et al. 1983). Northern pike, for example, often accumulate Hg to higher concentrations than other fishes of the same age in a given aquatic system (Phillips et al. 1980).

Summary

Dual composite samples of whole bluegills from five naturally acidic and five circumneutral lakes were analyzed for Al, Cd, Cu, Mn, Pb, V, and Zn. All bluegill samples analyzed were comprised of four-year-old fish, with the exception of the samples from one acidic lake, which contained six- and seven-year-old fish. Concentrations of Cd, Mn, Pb, V, and Zn in bluegills varied significantly among lakes, whereas concentrations of Al and Cu did not. Mean concentrations of Cd and Pb in bluegills from the ten lakes were inversely (rank) correlated with both pH and waterborne calcium concentration. Mean concentrations of Mn, V, and Zn in bluegills were unrelated to lake pH and did not exhibit the many-fold (~20X) variation among lakes shown by Cd and Pb. Internal concentrations of Mn, V, Zn, and other essential trace elements in fishes appear to be under greater homeostatic control than those of the nonessential elements Cd and Pb. The mean and maximum concentrations of Cd and Pb in whole bluegills from the acidic lakes were similar to, or exceeded, values reported for whole bluegills from U.S. lakes and rivers contaminated by metal-containing industrial effluents and urban runoff. In contrast, Cd and Pb concentrations in bluegills from the circumneutral Wisconsin lakes were similar to values reported for bluegills from uncontaminated waters.

Mercury concentrations were measured in axial (edible) muscle tissue of four-, five-, and seven-year-old walleyes from two acidic and three circumneutral lakes. Mercury concentrations in walleyes from the acidic lakes (range, 0.41-1.74 μg/g wet weight) were substantially higher than in walleyes from the circumneutral lakes (range, 0.22-0.57 μg/g wet weight). About one-third of the walleyes analyzed from the two acidic lakes contained Hg concentrations in excess of the 1.0 μg/g (wet weight) FDA "Action Level" for human consumption. Mercury concentrations were positively correlated with age and size of walleyes in acidic lakes and with age, but not size, of walleyes in circumneutral lakes. Given the remote location of the Wisconsin lakes, the close proximity and geologic similarity of the watersheds of the acidic and circumneutral lakes, and the absence of direct anthropogenic inputs of metals to the lakes, these findings suggest that Cd, Pb, and Hg in the acidic lakes are highly available to fish.

BILATERAL ASYMMETRY OF FISHES INHABITING ACIDIC AND CIRCUMNEUTRAL LAKES

In this chapter, measurements of fluctuating asymmetry of three meristic (countable and discrete), bilateral anatomical characters--numbers of pored lateral line scales, pectoral fin rays, and gill rakers on the outer arch--in fishes from naturally acidic and circumneutral study lakes are compared. The utility of fluctuating asymmetry as an indicator of pH-related developmental stress in fish is also discussed. Fluctuating asymmetry, the type of asymmetry studied here, is defined as deviation from perfect symmetry of any bilateral character (Valentine et al. 1973) and is considered to be nonadaptive (Van Valen 1962).

Prolonged exposure of certain teleosts to low pH can cause considerable developmental stress. The frequency of spinal deformities in the white sucker population in George Lake, Ontario, increased as the lake became progressively more acidic (Beamish et al. 1975). White suckers exposed to low pH in laboratory studies exhibited similar spinal deformities and other anatomical anomalies (Beamish 1972; Trojnar 1977). Beamish et al. (1975) hypothesized that the high incidence of spinal deformities in white suckers from acidified George Lake resulted from decalcification of bone. In a subsequent study of Ontario lakes, Fraser and Harvey (1982) found that the calcium content of centra was lower in white suckers from George Lake and two other acidic lakes (pH range, 4.65-5.36) than in fish from lakes with higher pH (range, 6.37-6.59); this was attributed to either active decalcification or reduced calcium deposition in bone during growth of fish in the acidic lakes. In a laboratory study, Nelson (1982) showed that the amount of calcium deposited in bone (ossification) in developing rainbow trout alevins was significantly less at pH 4.3 and 4.8 than at pH 7.3. The ability of rainbow trout alevins to ossify skeletal parts was unrelated to waterborne calcium concentration, which ranged from 5.5 to 112 mg/l in Nelson's study. Teleosts can mobilize calcium from internal reservoirs such as acellular bone and scales, when placed in a divalent ion-free environment (Fleming et al. 1973). Anatomical deformities have also been observed in fathead minnows *Pimephales promelas* (Mount 1973) and embryos and alevins of Atlantic salmon *Salmo salar* (Daye and Garside 1980) during long-term exposure to low pH in the laboratory.

In their detailed morphological study of Atlantic salmon, Daye and Garside (1980) noted that some structural injuries affected by acid stress were similar to those caused by toxic contaminants and thermal stress. Working with natural populations of three marine fishes, Valentine et al. (1973) found that temporal and spatial trends in fluctuating asymmetry of anatomical characters were best explained by environmental toxicant stress. They proposed that fluctuating asymmetry could be used to detect environmental stress induced by agents affecting the metabolism, transport, or deposition of calcium in fish. In a subsequent study, Valentine and Soule (1973) reported increasing asymmetry of pectoral fin rays of California grunion *Leuresthes tenuis* with increasing exposure concentrations of DDT in continuous flow bioassays. Ames et al. (1979) studied fluctuating asymmetry of centrarchids in impoundments in the southeastern United States and found the highest levels of asymmetry in fish collected from a naturally acidic, softwater pond.

Valentine (1975) studied anatomical deformities of barred sand bass *Paralabrax nebulifer* and found that the principal deformities encountered were related to calcified skeletal structures, including the gill rakers, fins, and vertebral column. Gill raker deformities were most prevelant and occurred in association with other internal and external skeletal anomalies in barred sand bass. The frequency and severity of anomalies of the gill rakers and other skeletal characters increased with age, and there was a strong association between skeletal deformities and levels of fluctuating asymmetry. Valentine hypothesized that the high incidence of skeletal deformities in barred sand bass and two other marine teleosts in coastal waters of southern California was due to toxicants that interfere with calcium metabolism. In a similar study, Slooff (1982) observed that bream *Abramis brama* had higher incidences of certain skeletal deformities in the Rhine River than in less polluted surface waters.

The use of fluctuating asymmetry of meristic anatomical characters as a measure of stress on fish populations in acidic surface waters was investigated because (1) low pH and elevated metal concentrations, two characteristics of acidified surface waters, are known to interfere with calcium metabolism in fish (Spry et al. 1981) and (2) levels of fluctuating asymmetry should be associated with other skeletal deformities of fish, such as spinal deformities (Valentine 1975). As a potential measure of stress, fluctuating asymmetry is attractive because it is relatively simple and economical to measure, and the technique can be applied to both museum specimens and recently collected specimens to evaluate temporal trends in asymmetry.

Methods

The bluegills and white suckers used in this analysis were collected with 91.4-m variable mesh gill nets and 0.95-cm mesh fyke nets from 8 July to 20 August 1980, during the comprehensive survey of fish communities in the study lakes. Fish were placed into plastic bags, held in ice-filled chests at the sampling site, and frozen (-4 C) within 12 hours of collection. After transport to the laboratory, fish were thawed, weighed to the nearest 0.1 g, and measured (total length) to the nearest millimeter. Bluegills used in this analysis were also analyzed for growth and condition, as described previously (Table 7). Age of bluegills was estimated by enumeration of scale annuli. Age of white suckers was not determined.

The following meristic anatomical characters were counted on the left and right sides of each fish: pored lateral line scales, pectoral fin rays, and gill rakers on the outer arch. Fish were examined under magnification, when necessary, and each count was repeated to ensure accuracy.

Data analyses were conducted with the Michigan Interactive Data Analyses System (MIDAS) at the University of Michigan, Ann Arbor (Statistical Research Laboratory 1976). The variance of the absolute difference between the left and right sides was used as the estimator of the amount of asymmetry of each meristic character in a given population (species and lake). This measure, V_a, is hereafter referred to as the "asymmetry variance." The arithmetic mean of the absolute difference in counts of a meristic character between the right and left sides in a given population is estimated by $\overline{d}$ and referred to as the "asymmetry mean." Statistical significance is defined as $P \leq 0.05$.

Results and Discussion

Bluegills from five naturally acidic and six circumneutral lakes were used in this analysis, because bluegills were not found in Zottle Lake (Table 3). Numbers and ranges of age and size of bluegills are given in Table 7.

Effects of age and lake category on asymmetry of the three meristic characters in bluegills were evaluated by one-way analysis of covariance (data from naturally acidic and circumneutral lakes were grouped as two separate treatments, with age of fish as a covariate). Age was not a significant covariate for any of the three meristic characters ($P > 0.30$ for all tests), and lake category was significant only for asymmetry of gill rakers ($P = 0.034$). The asymmetry mean of gill raker counts was greater in bluegills from the five acidic lakes ($\overline{d} = 0.54$) than in those from the six circumneutral lakes ($\overline{d} = 0.43$). Because asymmetry of the three meristic characters in bluegills was not related to age, all age classes were grouped in subsequent analyses.

Asymmetry means ($\overline{d}$) of each of the three meristic characters varied among lakes by less than a factor of three (maximum/minimum) in bluegills (Table 20). As expected, the two measures of asymmetry ($\overline{d}$ and V_a) were strongly rank correlated. The Spearman correlation coefficient (r_s) between $\overline{d}$ and V_a was 0.95 ($P < 0.001$; one-tailed test for positive correlation) for pored lateral line scales, 0.84 ($P < 0.001$) for pectoral fin rays, and 0.80 ($P < 0.005$) for gill rakers on the outer arch. Hereafter, the asymmetry variance, V_a, will be used as the singular measure of asymmetry, as recommended by Valentine and Soule (1973).

Table 20. Measures of asymmetry of three meristic, anatomical characters in bluegills from eleven lakes in northern Wisconsin. Lakes are listed vertically in order of increasing pH.

Lake category and name	pH	Pored lateral line scales		Pectoral fin rays		Gill rakers on the outer arch	
		$\bar{d}$	V_a	$\bar{d}$	V_a	$\bar{d}$	V_a
Acidic							
Hilderbrand	5.1	2.19	28.7	0.12	0.11	0.67	0.42
Lumen	5.2	2.33	4.23	0.24	0.19	0.52	0.42
Currie	5.6	1.29	1.45	0.24	0.23	0.48	0.38
Flannery	5.7	1.88	3.30	0.20	0.20	0.57	0.61
Clara	6.0	1.49	1.81	0.32	0.34	0.54	0.38
Circumneutral							
Toulish	6.7	1.66	2.74	0.17	0.14	0.54	0.34
Hixon	6.8	2.81	10.0	0.20	0.28	0.28	0.24
Clear	6.9	1.65	2.39	0.18	0.15	0.46	0.25
Dorothy Dunn	6.9	1.00	0.60	0.19	0.16	0.38	0.25
Nelson	7.0	1.89	4.71	0.19	0.28	0.33	0.26
Garth	7.5	1.64	2.66	0.33	0.39	0.55	0.34

Rank correlation analyses (one-tailed tests for negative correlation) of lake pH and asymmetry variances for bluegills yielded results similar to those obtained with analysis of covariance. Lake pH and V_a were significantly and negatively correlated for gill rakers (r_s = -0.72, $P < 0.01$), but not for pored lateral line scales (r_s = -0.24) or pectoral fin rays (r_s = 0.43). Although the asymmetry of gill rakers increased with decreasing pH in these lakes, the overall asymmetry mean for gill rakers of bluegills from the five acidic lakes exceeded that for the six circumneutral lakes only by about 25%. The asymmetry variance for gill rakers was also negatively correlated with mean calcium concentration during summer (r_s = -0.67, $P < 0.025$). Asymmetry variances for pored lateral line scales and pectoral fin rays were unrelated to waterborne calcium concentrations in these lakes.

The relation between asymmetry variances of the three meristic characters and relative density of bluegill populations was also examined. Numbers of bluegills captured in fyke and gill nets (Table 7) were used to rank relative population density in the eleven lakes. The Spearman correlation coefficient between density and V_a was significant for pectoral fin rays (r_s = 0.625, $P < 0.025$; one-tailed test for positive correlation), but not for gill rakers (r_s = 0.05) or pored lateral line scales (r_s = 0.35).

White suckers were collected in sufficient numbers for analysis ($n \geq 15$) in only four lakes--two acidic and two circumneutral (Table 21). Asymmetry variances for pectoral fin rays in white suckers were greater in the two acidic lakes than in the circumneutral lakes; however, a significant correlation with pH cannot be stated because of the small number of lakes. No potential positive relation between lake pH and fluctuating asymmetry of either pored lateral line scales or gill rakers was apparent for white suckers (Table 21).

Results of this analysis of fluctuating asymmetry as a potential measure of pH-related stress were largely inconclusive in the pH range studied here. Fluctuating asymmetry of the meristic characters measured in bluegills and white suckers from northern Wisconsin lakes were either unrelated or only marginally related to pH. Ames et al. (1979) reported relatively high levels of fluctuating asymmetry of a number of anatomical characters in bluegills from Skinface Pond, a highly organic softwater impoundment in South Carolina. Skinface Pond has an annual pH range of 4.2-5.0 and a mean pH of about 4.6 (Wiener and Giesy 1979), which represents a mean hydrogen ion concentration more than three-fold greater than that in the lakes studied here (Table 20). Exposure of fish to high waterborne concentrations of certain metals (e.g., Cd, Pb, Hg, and Zn) can also induce skeletal deformities (Valentine 1975; Slooff 1982). Concentrations of these metals are often elevated in surface waters undergoing cultural acidification. It is therefore tempting to speculate that a significant relation between pH and fluctuating asymmetry of fish populations could occur in culturally acidified lakes. However, Jagoe (1983) found that asymmetries of three fishes in lakes of the northeastern United States, with pHs ranging from 4.30 to 6.77, were only weakly related to stress associated with acidification. Fluctuating asymmetries of a few anatomical characters in each species

Table 21. Measures of fluctuating asymmetry of three meristic, anatomical characters in white suckers from four lakes in northern Wisconsin.

Lake	pH	Wet weight of fish analyzed (g)			Pored lateral line scales		Pectoral fin rays		Gill rakers on the outer arch	
		n	Mean	Range	$\bar{d}$	V_a	$\bar{d}$	V_a	$\bar{d}$	V_a
Currie	5.6	49	466	259-621	2.73	25.1	0.47	1.17	1.14	0.96
Zottle	5.6	15	922	769-1069	1.71	1.45	0.33	0.38	1.33	0.52
Clear	6.9	43	563	42-1151	1.89	6.52	0.37	0.29	1.07	1.24
Dorothy Dunn	6.9	16	480	101-1235	2.00	2.00	0.25	0.20	0.93	1.30

were greater in acidified lakes, but asymmetries of most characters were not (Jagoe 1983).

Barahona-Fernandes (1982) postulated that the majority of anatomical abnormalities appearing in larval European sea bass *Dicentrarchus Labrax* were lethal, whereas abnormalities appearing in post-larval forms did not influence survival. The effects of asymmetry of the meristic characters studied here on the survival of developing bluegills and white suckers are not known. Valentine et al. (1973) observed increasing asymmetry of pectoral fin rays and gill rakers with increasing standard length of barred sand bass from coastal waters of southern California. In contrast, asymmetry of pectoral fin rays, gill rakers, and pored lateral line scales in bluegills from Wisconsin lakes were unrelated to age.

The ability to detect asymmetry in natural populations of adult fishes (studied here) will be influenced by differential mortality rates among fish with varying levels of asymmetry. Fluctuating asymmetry in fish may be positively correlated with other skeletal anomalies (Valentine 1975) that do affect survival. High mortality of abnormal fishes during early life stages would also be consistent with results of Daye and Garside (1980) and Trojnar (1977). Analysis of developmental abnormalities in early life history stages may provide a better estimate of developmental stress on fish populations than measures of fluctuating asymmetry of adult fish.

Summary

Fluctuating asymmetry of bilateral anatomical characters, which was previously proposed as an indicator of stress induced by agents affecting calcium metabolism of fishes, was measured in bluegills from eleven lakes and white suckers from four lakes in north central Wisconsin. In general, fluctuating asymmetry of three meristic characters--numbers of pored lateral line scales, pectoral fin rays, and gill rakers on the outer arch--were either unrelated or only marginally related to either lake pH or waterborne calcium concentration in these species. In bluegills, asymmetry variance of gill raker counts was negatively correlated with both lake pH and waterborne calcium concentration; however, the overall asymmetry mean of gill raker counts in bluegills from the naturally acidic lakes only slightly exceeded that in bluegills from the circumneutral lakes. In white suckers, asymmetry variances of pectoral fin ray counts were greater in fish from two naturally acidic lakes than in those from two circumneutral lakes, but a significant relation to pH could not be established.

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RECOMMENDATIONS FOR FUTURE RESEARCH

These studies indicate that the chemical conditions in the naturally acidic lakes studied here (pH range 5.1-6.0) were stressful to certain fish taxa. Acid-sensitive taxa were rare or absent in the naturally acidic lakes, but were generally well represented in the circumneutral lakes. It was hypothesized that serum calcium concentrations in female white suckers prior to spawning were depressed in lakes with autumnal pH of 5.6 or lower and waterborne calcium concentrations of 2.0 mg/l or less. Accumulation of the toxic metals Cd, Pb, and Hg by fish was substantially greater in the naturally acidic than in the circumneutral lakes.

Fishery biologists are presently without proven diagnostic tools for detecting stress on fish populations in acidifying lakes *before* the onset of recruitment failure (Mills 1983). Development of indicators of *chronic*, pH-related stress on fish populations is therefore needed. Analysis of growth and condition of bluegills revealed that these parameters are of little value for assessing chronic, pH-related stress on fish species with density-dependent growth. These results are consistent with those of Mills (1983 and personal communication), who found that reductions in growth rates of fishes in Lake 223 of the Experimental Lakes Area (Ontario) did not occur until recruitment failures had already begun. Fluctuating asymmetry of anatomical characters, proposed by Valentine et al. (1973) as an indicator of stress induced by agents affecting calcium metabolism, in bluegill and white sucker populations was unrelated or only weakly related to pH in the Wisconsin study lakes. Jagoe's (1983) study of fluctuating asymmetry of fishes in acidified lakes in the northeastern United States yielded similar results; consequently, fluctuating asymmetry appears to have little potential as an indicator of pH-related stress on fish populations. In contrast, the utility of serum calcium concentrations, measured prior to spawning, as a measure of pH-related stress on populations of white suckers and other fish species with synchronous reproductive cycles merits further study. Measurement of erythrocyte δ-amino levulinic acid dehydratase (ALA-D) activity in fishes from naturally acidic Wisconsin lakes would be useful for evaluating chronic stress associated with increased body burdens of Pb (Hodson et al. 1977).

Wood (1974) classified Cd, Pb, and Hg as "very toxic and relatively accessible" elements. The data presented herein on Cd, Pb, and Hg in fish suggest that acidification of susceptible Wisconsin lakes would result in increased accumulation of these metals by fish. Relatively small decreases in pH (0.1-0.2 units) can substantially alter the aqueous speciation of certain metals, and it would not be unreasonable to suggest that the first quantifiable (by monitoring) effect on fish stocks in susceptible Wisconsin lakes--given an acidification scenario--would be elevated concentrations of Cd, Pb, and Hg. Mercury contamination of fish stocks to levels above the U.S. Food and Drug Administration's action level could adversely affect the sport fishing industry in Wisconsin.

The effects of metal-contaminated fish stocks in this area could also extend to fish-eating birds. During the nesting season, many lakes in the study area are inhabited by common loons (*Gavia immer*), which feed primarily on fish. The potential effects of consumption of metal-contaminated fish on common loon populations merit investigation, given the aesthetic importance of this species.

A number of attributes of fishery resources in northern Wisconsin lakes (e.g., species richness and composition, metal contamination, and perhaps serum calcium levels in synchronous spawners) exhibit gradients in apparent response to variations among lakes in pH, waterborne calcium concentration, or both. The ranges of waterborne calcium concentrations did not overlap between the naturally acidic and circumneutral lakes studied here. Consequently, it is difficult to distinguish between the effects of pH and waterborne calcium with the existing field data. Density-dependent gradients in attributes such as growth and condition of certain fishes are also evident in northern Wisconsin lakes. The existence of these gradients will complicate acidification-related assessments and both temporal and spatial comparisons of fishery resources in these lakes. Other, extrinsic factors can also complicate attempts to associate temporal shifts in lacustrine fish populations and communities with changes in water chemistry. Interim changes in a variety of factors, such as weather, stocking practises, fishing pressure, and interspecific interactions among fish populations, can greatly complicate determination of cause and effect relationships in field studies (e.g., Inskip and Magnuson 1983). Stress associated with increased waterborne concentrations of toxic metals can result in subtle fish population responses that are difficult to detect (McFarlane and Franzin 1978). Surveillance of fish communities may be more reliable than intensive monitoring of single species populations for detecting *acute* stress associated with acidification (Harvey 1982; Schindler 1983).

The distributions of the prevalent fish species in northern Wisconsin lakes, relative to low pH, have been quantified in field studies (Rahel and Magnuson 1983). However, corresponding laboratory data on the chronic toxicity of pH are not available for most of these taxa (Marcus et al. 1983). Reliable predictions of the potential effects of acidification on these fishery resources will therefore require additional information on the toxicity of low pH to sensitive life stages across the range of waterborne calcium concentrations occurring in susceptible Wisconsin lakes. However, it should be recognized that the minimum pH limits for fishes may be lower in the laboratory than in the field (Peterson et al. 1982). Predictions of the mobilization of potentially toxic metals, such as Al, into the waters of these lakes must also be made to accurately assess the potential effects of acidification on fishery resources (Baker and Schofield 1982).

In Scandinavia and eastern North America, acidification of sensitive surface waters has been detected within one to two decades where precipitation pH was less than 4.6 (Committee on the Atmosphere and the Biosphere 1981). The annual, volume-weighted mean pH of precipitation in north central Wisconsin is near this apparent threshold value. Consequently, it is important that dose-response models, which are applicable to sensitive drained and seepage lakes in this area, be developed and validated. Acidification models developed for watersheds containing shallow, impervious bedrock will not likely be applicable to watersheds in the Highland Lake District of Wisconsin, because of their hydrologic and geologic dissimilarities.

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Appendix 1. Additional descriptions of the twelve study lakes in northern Wisconsin.

Lake category and name	Location[a]				Watershed[b] area (ha)		Adjoining wetlands (ha)	Land use in watershed (% of land area)[c]						Human development (summer 1980)		Additional surface water resources descriptions[e]
	County	T	R	S	Total	Immed.		Upland deciduous	Upland conifer	Open field	Agricultural	Swamp conifer	Open bog	Private cabins	Resorts[d]	
Acidic																
Clara	Lincoln	35	7	14	164.8	92.4	8.1	45	32	0	10	13	0	9	1(5)	Littoral bottom materials consist primarily of sand (60%), muck (25%), and gravel (15%). Submergent vegetation of moderate density occurs in 65% of the lake basin and floating vegetation is dense over 30%. Public access is available.
Currie	Oneida	37	6	14	148.7	148.7	21.0	68	7	0	0	12	13	37	0	Gravel (35%), sand (25%), and rubble (20%) are the predominant littoral materials with muck (15%) and some boulders present. Floating vegetation is dense in portions of the basin with submergent vegetation present in moderate density. No public access.
Flannery	Oneida	37	8	28	100.5	75.4	5.3	81	14	0	0	5	0	81	0	Sand is the predominant littoral material (70%) with muck (20%), gravel, rubble, and boulders present. Public access is available.
Hilderbrand	Lincoln	35	8	1	114.7	84.0	12.1	59	7	0	0	34	0	12	0	Littoral materials consist of muck (35%), sand (25%), rubble (20%), gravel (15%), and boulders (5%). No public access.
Lumen	Oneida	38	7	33	49.3	49.3	6.5	50	19	0	0	13	19	5	0	Sand (45%) and muck (45%) are the principal littoral materials with some gravel present. Public access is of the wilderness type.
Zottle	Oneida	39	7	34	56.6	51.1	0.4	70	26	0	0	0	4	0	0	Sand is the predominant littoral material (65%) with rubble (20%), gravel (10%), and some muck present. Public access is available.
Circumneutral																
Clear	Oneida	36	9	10	42.9	42.9	0	57	43	0	0	0	0	12	1	Sand is the principal littoral material (75%) with gravel (20%) and some rubble present. No public access.

Appendix 1. Continued.

Lake category and name	Location[a]				Watershed[b] area (ha)		Adjoining wetlands (ha)	Land use in watershed (% of land area)[c]						Human development (summer 1980)		Additional surface water resources descriptions[e]
	County	T	R	S	Total	Immed.		Upland deciduous	Upland conifer	Open field	Agricultural	Swamp conifer	Open bog	Private cabins	Resorts[d]	
Dorothy Dunn	Vilas	42	8	26	95.3	95.3	6.1	82	15	0	0	0	3	0	0	Bottom materials consist primarily of sand, with gravel and muck present. The lake is presently maintained as a wilderness lake. Beaver and muskrats are present. The lake is used by puddle ducks and to a greater extent by diving ducks during spring migration. The entire shoreline is in public ownership.
Garth	Oneida	38	6	28	110.2	101.5	9.7	47	39	0	0	8	5	48	1(21)	Sand is the principal littoral material (60%) with muck (35%) and some gravel present. Submergent vegetation is dense. Public access is of the wilderness type. Black ducks and wood ducks use this lake as a nesting site. Puddle ducks use this lake during spring and fall migrations. Diving ducks use this lake during fall migrations.
Hixon	Oneida	36	9	30	178.7	110.8	3.2	44	29	0	9	18	0	30	0	Sand is the predominant littoral material (65%) with muck (25%) and some gravel present. No public access.
Nelson	Vilas	42	6	31	54.2	53.6	0	68	24	4	0	0	4	13	0	Bottom materials consist primarily of sand and some gravel. Public access is of a difficult or unimproved nature.
Toulish	Vilas	40	6	6	71.4	71.4	6.1	83	4	0	0	9	4	11	0	Bottom materials consist chiefly of sand with gravel and some rock and muck present. No public access.

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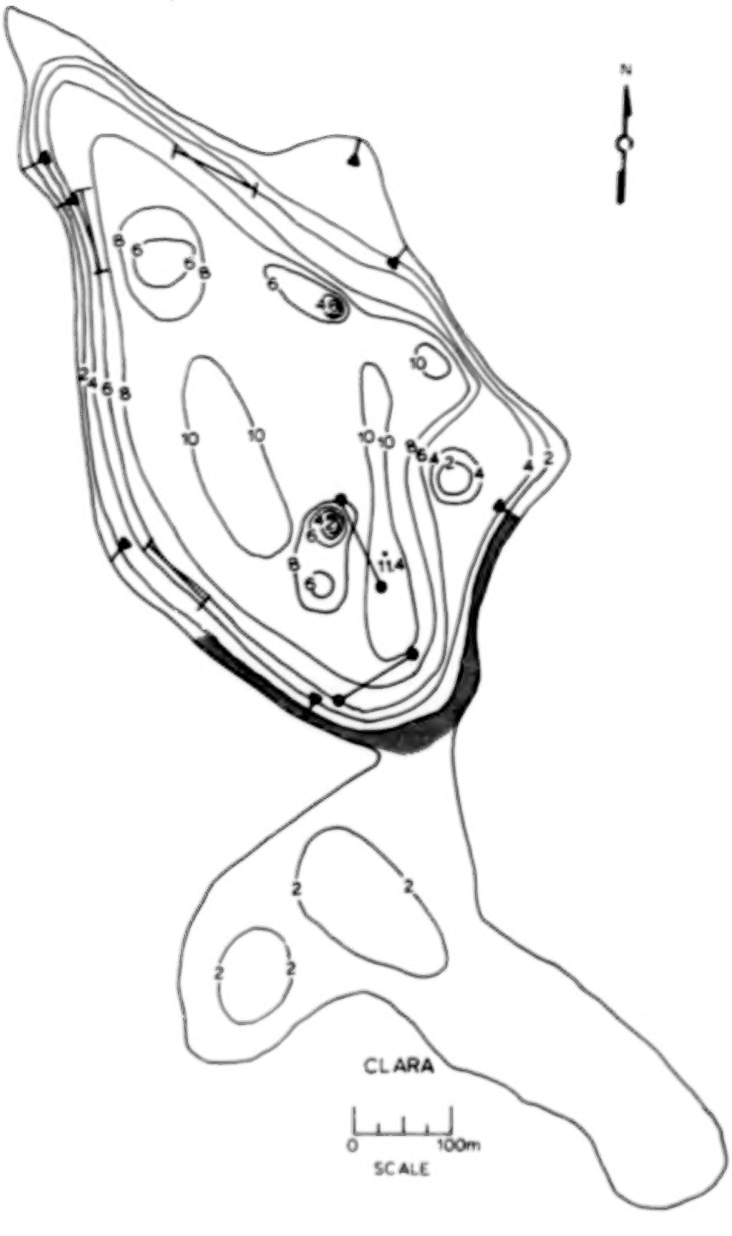
[a] T = township, R = range, S = section.

[b] Area of immediate watershed includes lake area and land area draining directly into the lake. Total watershed area includes lake area, immediate watershed area, and additional land and water surface area contributing flow to the lake. Data provided by K. E. Webster (Wisconsin Department of Natural Resources, Rhinelander).

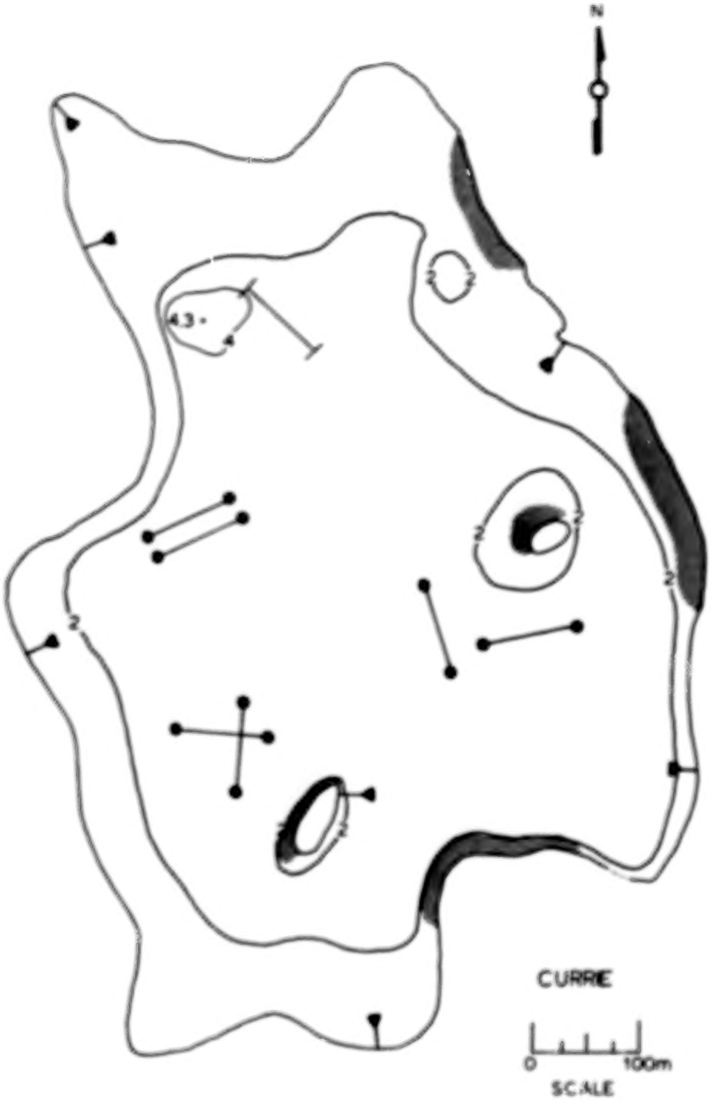
[c] Data provided by K. E. Webster (Wisconsin Department of Natural Resources, Rhinelander).

[d] Number of resort cabins is given in parentheses.

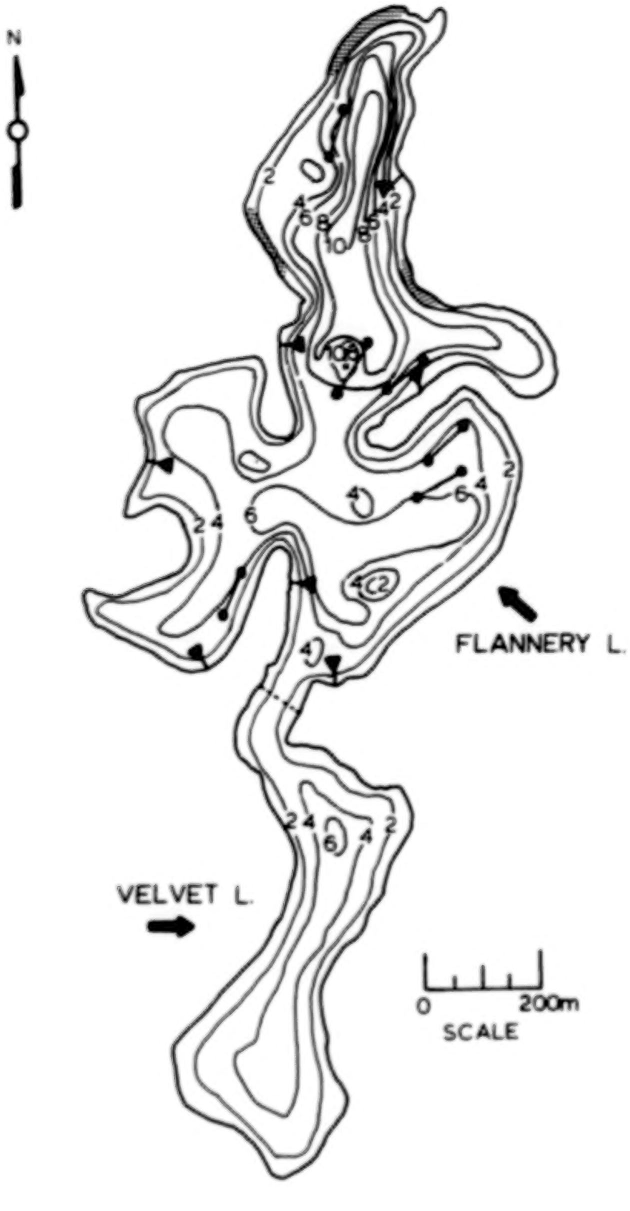
[e] Modified from descriptions of lakes in Wisconsin Department of Natural Resources Surface Water Resources handbooks for Lincoln (Carlson and Andrews 1982), Oneida (Andrews and Threinen 1966), and Vilas (Black et al. 1963) counties.



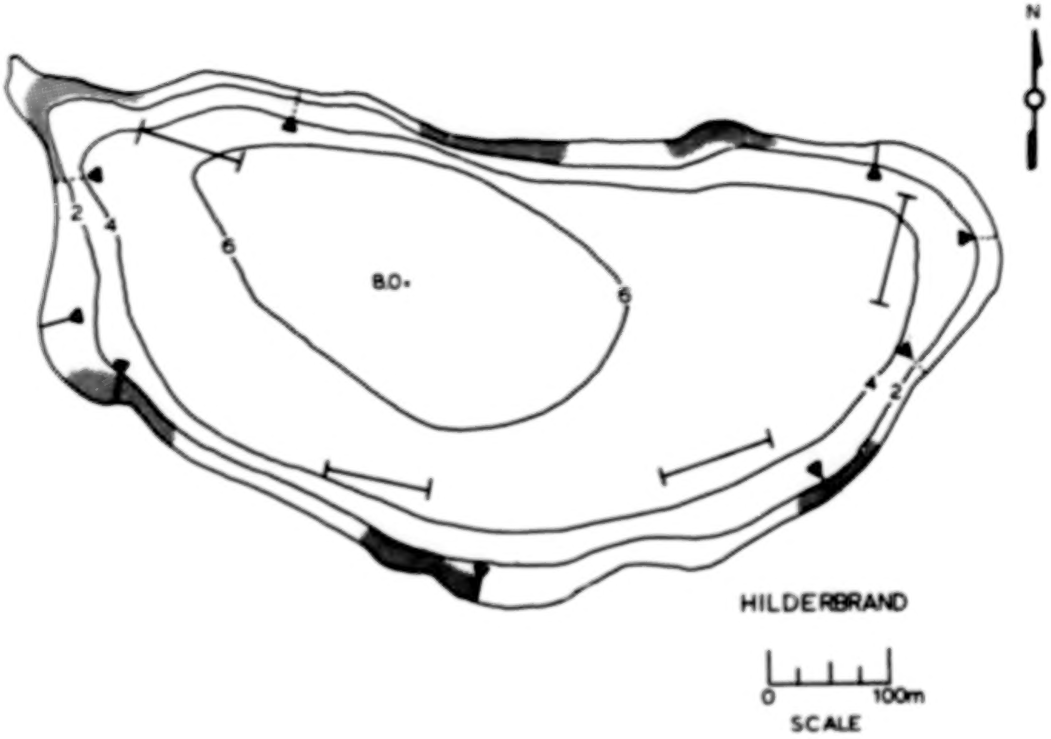
Appendix 2. Morphometric map of Clara Lake with depth contours in meters. Triangles with lines to shoreline indicate locations of fyke net sets for two nights, lines with bars at each end indicate locations of gill net sets for two nights, lines with closed circles at each end indicate locations of gill net sets for one night, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, August 4-5, 1980.



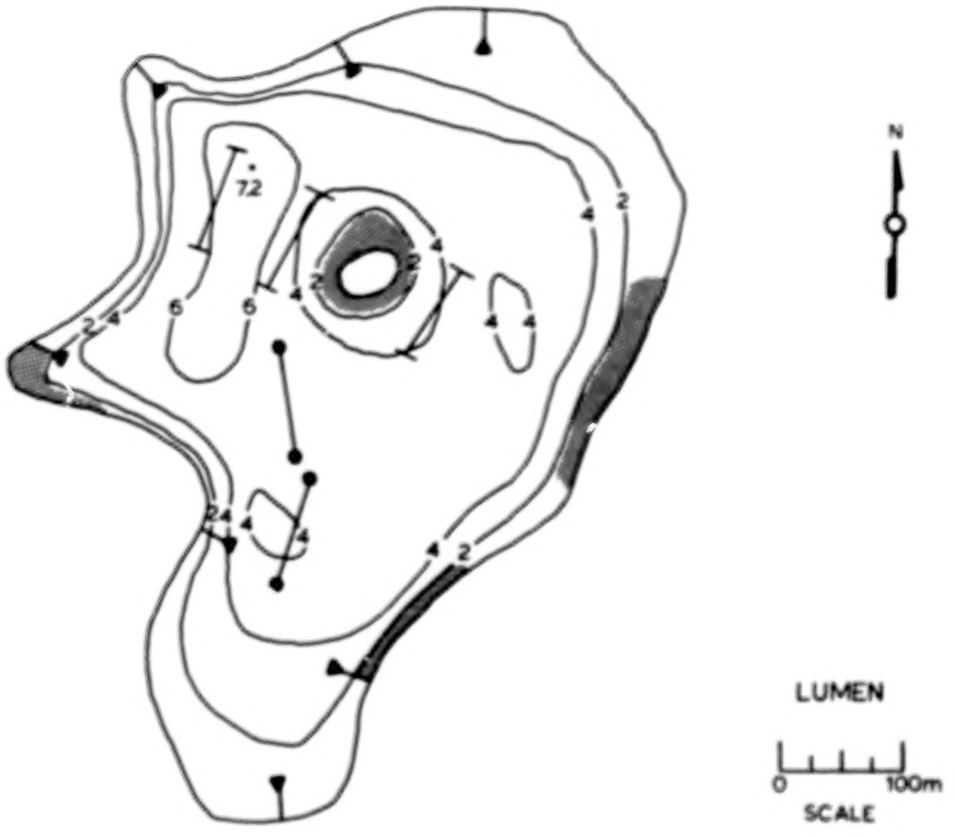
Appendix 3. Morphometric map of Currie Lake with depth contours in meters. Triangles with lines to shoreline indicate locations of fyke net sets for two nights, lines with bars at each end indicate locations of gill net sets for two nights, lines with closed circles at each end indicate locations of gill net sets for one night, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, July 16-17, 1980.



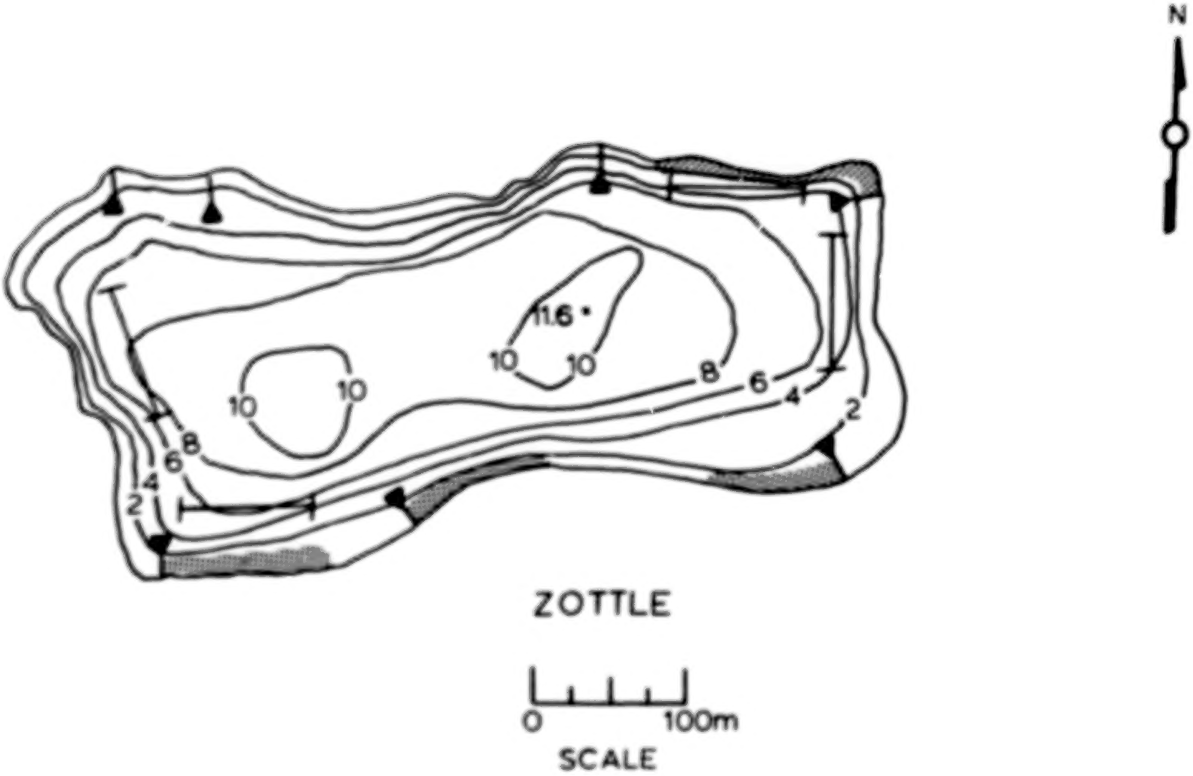
Appendix 4. Morphometric map of Flannery Lake with depth contours in meters. Triangles with lines to shoreline indicate locations of fyke net sets for two nights, lines with closed circles at each end indicate locations of gill net sets for one night, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, July 8-10, 1980.



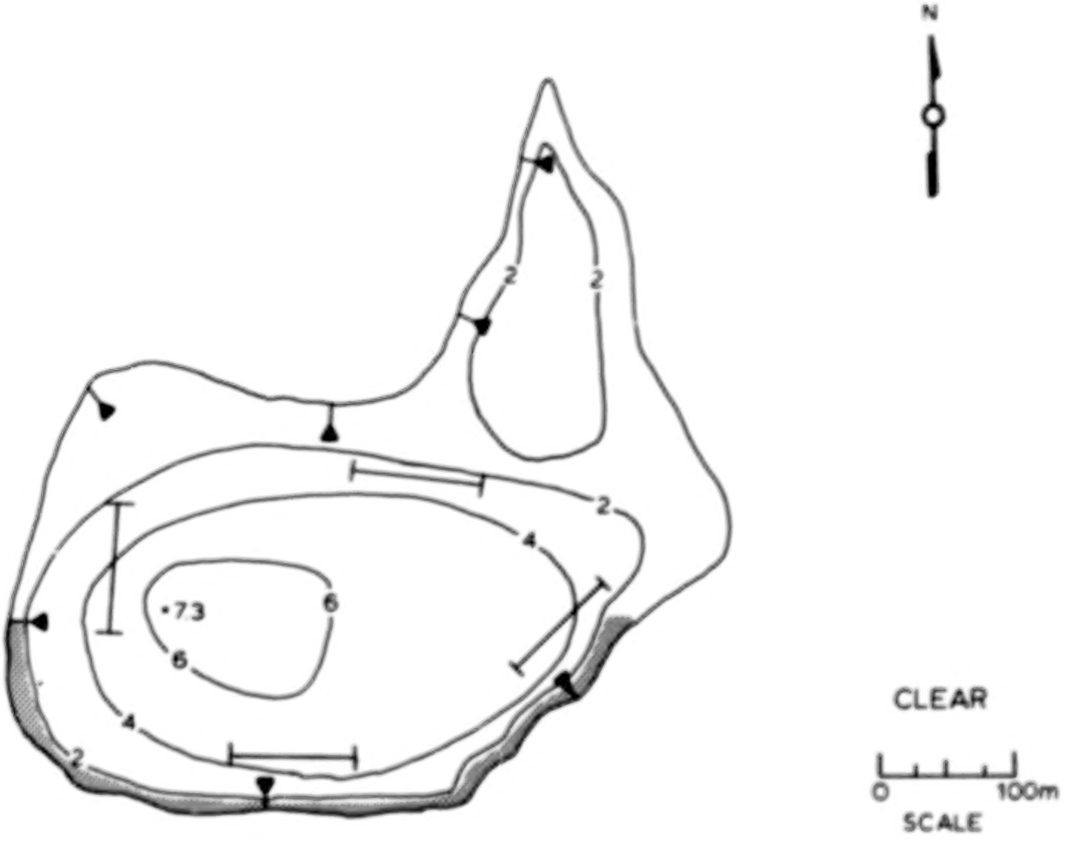
Appendix 5. Morphometric map of Hilderbrand Lake with depth contours in meters. Triangles with solid lines to shoreline indicate locations of fyke net sets for two nights, triangles with dashed lines to shoreline indicate locations of fyke net sets for one night, straight lines indicate locations of gill net sets for two nights, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, July 21-22, 1980.



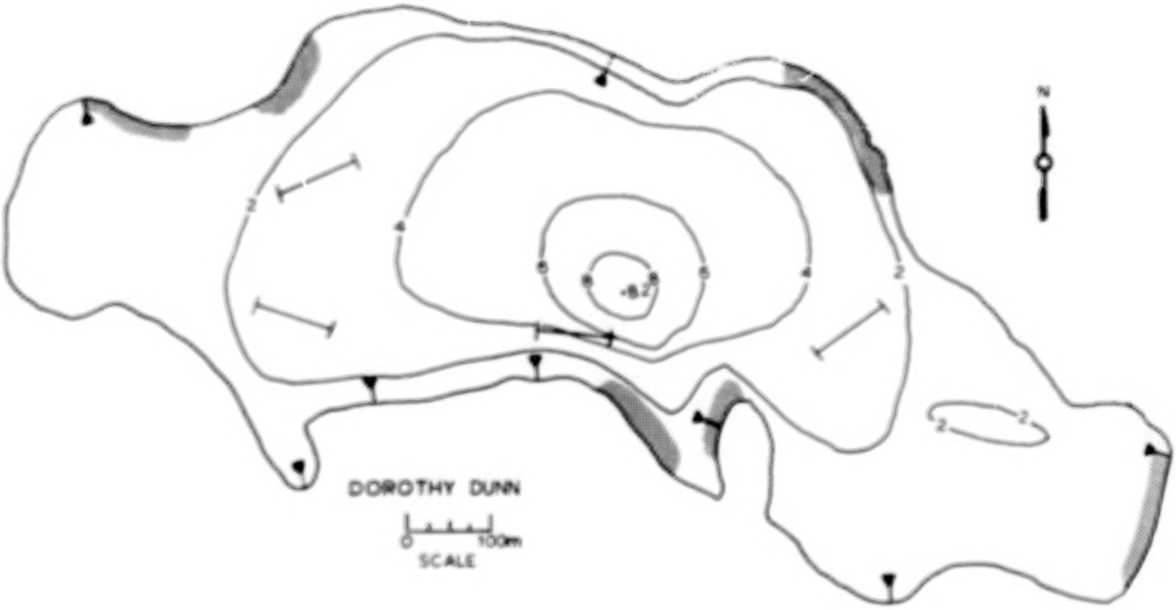
Appendix 6. Morphometric map of Lumen Lake with depth contours in meters. Triangles with lines to shoreline indicate locations of fyke net sets for two nights, lines with bars at each end indicate locations of gill net sets for two nights, lines with closed circles at each end indicate locations of gill net sets for one night, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, July 14-15, 1980.



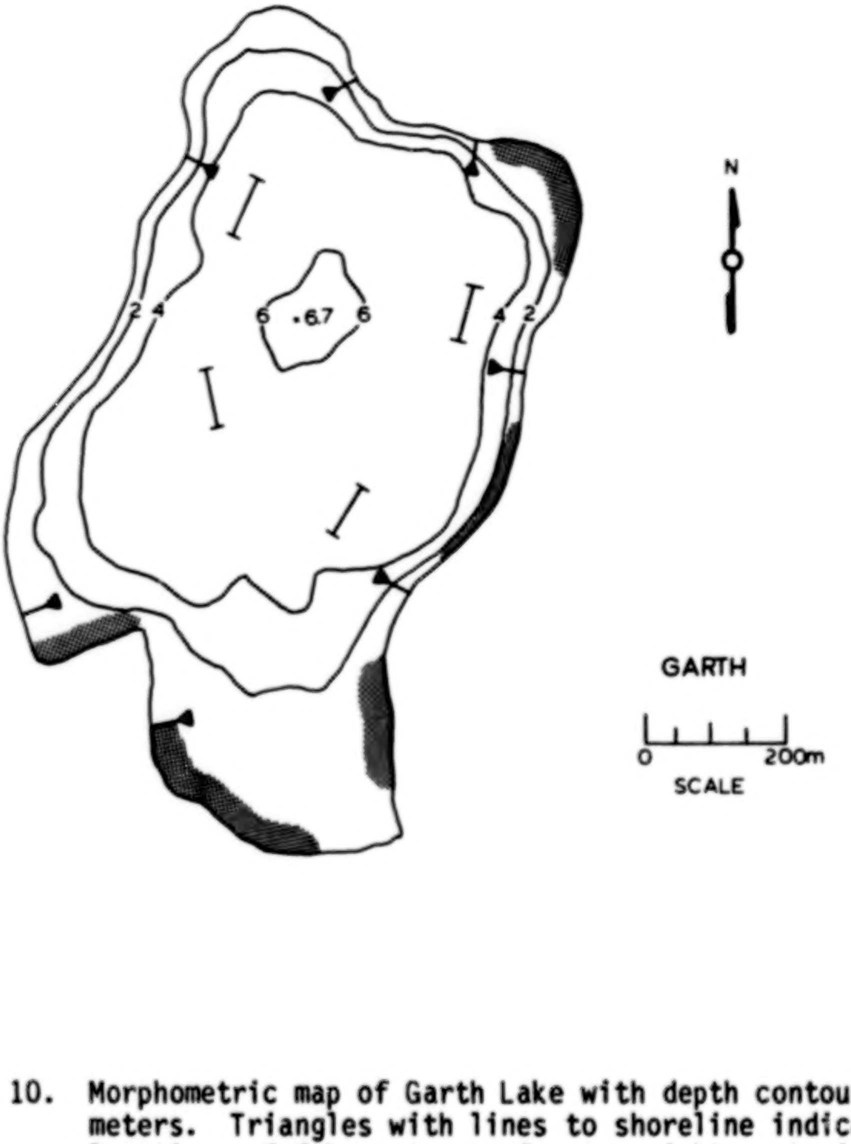
Appendix 7. Morphometric map of Zottle Lake with depth contours in meters. Triangles with lines to shoreline indicate locations of fyke net sets for two nights, straight lines indicate locations of gill net sets for two nights, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, August 11-12, 1980.



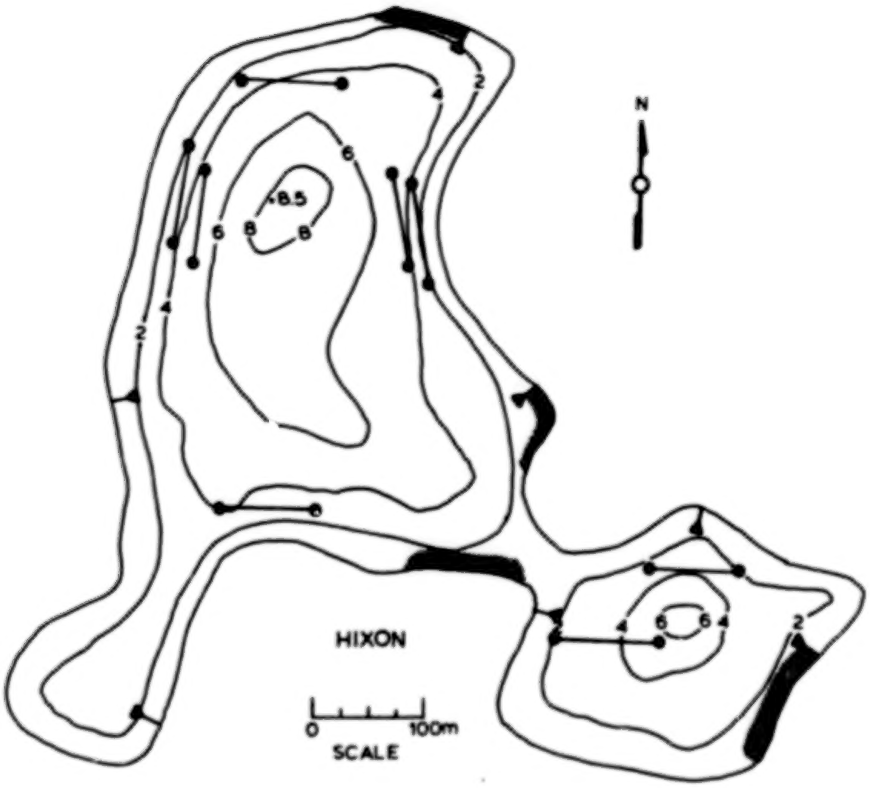
Appendix 8. Morphometric map of Clear Lake with depth contours in meters. Triangles with lines to shoreline indicate locations of fyke net sets for two nights, straight lines indicate locations of gill net sets for two nights, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, July 30-31, 1980.



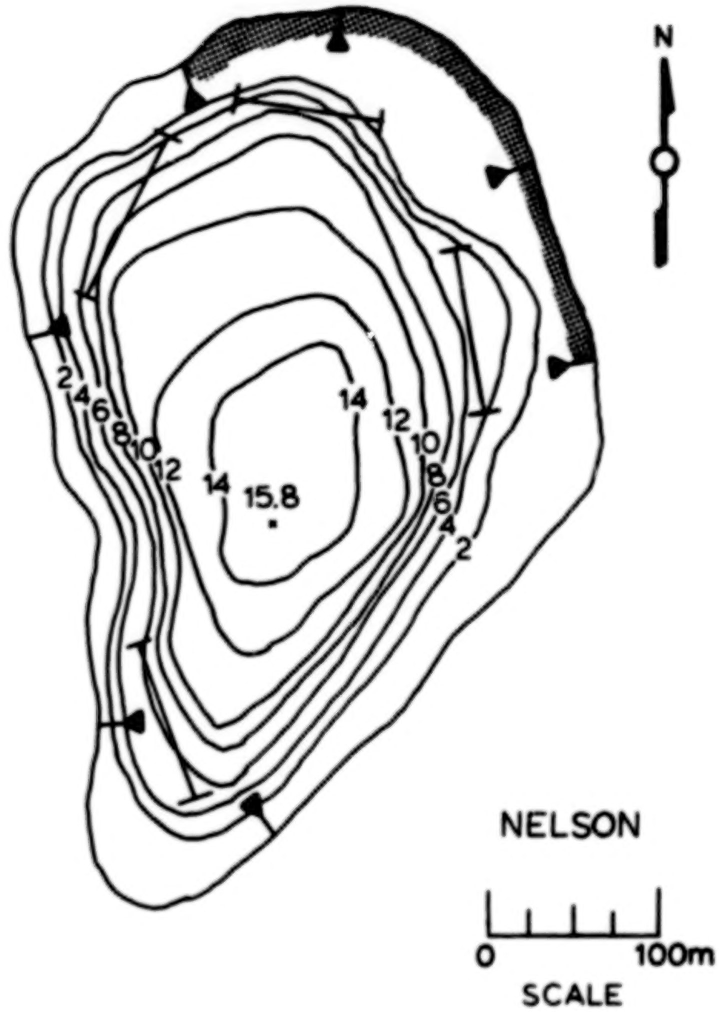
Appendix 9. Morphometric map of Dorothy Dunn Lake with depth contours in meters. Triangles with solid lines to shoreline indicate locations of fyke net sets for two nights, triangles with dashed lines to shoreline indicate locations of fyke net sets for one night, straight lines indicate locations of gill net sets for two nights, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, July 28-29, 1980.



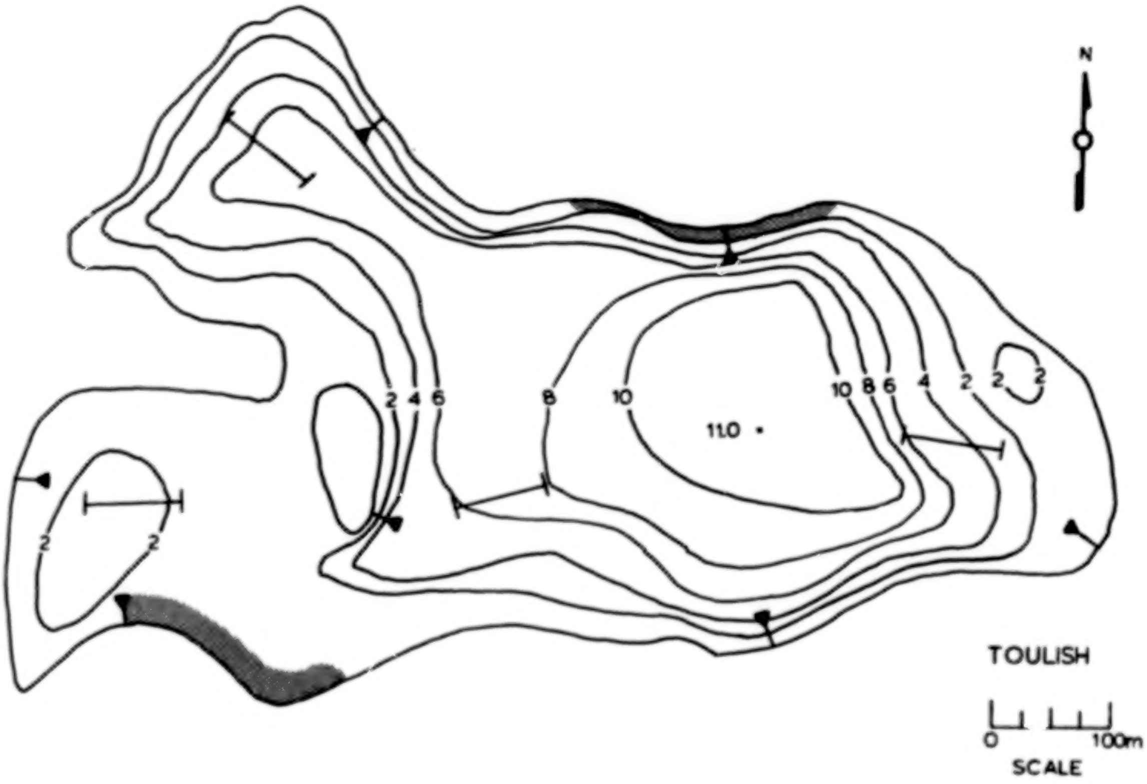
Appendix 10. Morphometric map of Garth Lake with depth contours in meters. Triangles with lines to shoreline indicate locations of fyke net sets for two nights, straight lines indicate locations of gill net sets for two nights, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, August 6-7, 1980.



Appendix 11. Morphometric map of Hixon Lake with depth contours in meters. Triangles with lines to shoreline indicate locations of fyke net sets for two nights, lines with closed circles at each end indicate locations of gill net sets for one night, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, July 23-24, 1980.



Appendix 12. Morphometric map of Nelson Lake with depth contours in meters. Triangles with lines to shoreline indicate locations of fyke net sets for two nights, straight lines indicate locations of gill net sets for two nights, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, August 18-19, 1980.



Appendix 13. Morphometric map of Toulish Lake with depth contours in meters. Triangles with lines to shoreline indicate locations of fyke net sets for two nights, straight lines indicate locations of gill net sets for two nights, and shaded areas indicate locations of shoreline seining during the comprehensive fishery survey, August 13-14, 1980.

Appendix 14. Calcium concentrations in waters (0.5 m below surface) of naturally acidic and circumneutral lakes in north central Wisconsin. All calcium determinations were made by atomic absorption spectrophotometry.

	Calcium concentration (mg/l)						
Lake category and name	Summer 1979[a]	June 1982[b]	August 1982[b]	October 1982[b]	January 1983[b]	June 1983[b]	Mean calcium conc. during summer (mg/l)[c]
Acidic							
Clara	1.8	1.6	1.6	2.6	2.6	1.9	1.7
Currie	1.1	0.7	1.0	1.6	2.1	1.0	1.0
Flannery	1.6	1.0	1.2	2.6	1.9	1.5	1.3
Hilderbrand	1.3	1.0	1.1	2.3	1.6	1.4	1.2
Lumen	1.2	0.7	0.9	2.8	2.0	1.0	1.0
Zottle	1.2	0.7	0.8	3.5	1.3	1.1	1.0
Range	1.1-1.8	0.7-1.6	0.8-1.6	1.6-3.5	1.3-2.6	1.0-1.9	1.0-1.7
Circumneutral							
Clear	--	7.9	8.8	8.4	8.2	7.8	8.2
Dorothy Dunn	--	4.0	4.5	4.7	4.9	4.1	4.2
Garth	9.6	9.9	11.3	11.0	9.6	9.5	10.1
Hixon	--	4.4	5.0	5.6	4.7	4.7	4.7
Nelson	--	4.3	5.0	5.9	4.7	4.6	4.6
Toulish	--	5.2	6.0	6.2	5.3	5.9	5.7
Range	--	4.0-9.9	4.5-11.3	4.7-11.0	4.7-9.6	4.1-9.5	4.2-10.1

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[a] Source: Eilers et al. (in press); samples were collected during July and August of 1979.

[b] Source: P. S. Schmidt, J. G. Wiener, D. E. Powell, and R. G. Rada (unpublished data).

[c] Means of values for summer 1979, June 1982, August 1982, and June 1983.

Appendix 15. Summary of available historical data on fish populations from Wisconsin Department of Natural Resources lake files. No historical fish survey data were available for Clear, Currie, Hixon, Lumen, Toulish, and Zottle Lakes. Species are grouped by family.

	Hilderbrand Lake	Flannery Lake		Clara Lake		Dorothy Dunn Lake	Nelson Lake		Garth Lake
Species	1958[a]	1959[b]	1967[c]	1953[d]	1969[e]	1944[c]	1950[f]	1962[g]	1950[h]
Northern pike	--	--	--	1	4	--	--	--	38
Muskellunge	--	--	--	--	--	2	2	2	--
Golden shiner	--	--	--	--	--	1	--	--	--
White sucker	--	--	--	--	1	1	--	--	2[i]
Black bullhead	--	--	common-abundant	--	--	--	--	--	--
Yellow bullhead	--	--	--	--	70	--	--	--	--
Bullhead[j]	21	--	--	--	--	--	--	--	95
Rock bass	--	--	--	--	--	--	24	4	17
Pumpkinseed	--	--	present	--	4	--	28[k]	7[k]	377[k]
Bluegill	--	846	common	237	644	51	61	16	1,609
Smallmouth bass	--	--	--	--	--	--	19	--	--
Largemouth bass	52	16	common	11	2	9	--	14	65
Black crappie	--	6[l]	present	3[l]	1	--	79[l]	--	285[l]
Warmouth	--	--	present	--	--	--	--	--	--
Yellow perch	37	1,997	common	--	1	6	13	8	11
Walleye	--	--	--	--	--	--	5	--	1

[a] Three 1-inch mesh fyke nets and two 1/4-inch mesh fyke nets set for three days, July 15-17, 1958

[b] Unknown number of gill nets set for four days during April 1959.

[c] Sampling effort and specific dates not included in file.

[d] Unknown number of seine hauls with an 800-foot seine on July 23, 1953

[e] Four 1-inch mesh fyke nets and two 1/4-inch mesh fyke nets set for three days at depths of 4-9 feet, June 24-27, 1969.

[f] Two 1-inch mesh fyke nets and three 1/4-inch mesh fyke nets set for three days, July 5-8, 1950.

[g] Electrofishing twice around the lake on July 20, 1962.

[h] Four 1-inch mesh fyke nets and two 1/4-inch mesh fyke nets set for three days, May 31-June 2, 1950.

[i] Listed in data file as "sucker"; assumed to be white sucker.

[j] Genus *Ictalurus*; assumed to be either black or yellow bullhead.

[k] Listed in data file as "sunfish"; assumed to be pumpkinseed.

[l] Listed in data file as "crappie"; assumed to black crappie.

Appendix 16. Historical records of fish stocking in the study lakes, compiled from Wisconsin Department of Natural Resources lake files. The files for Clear, Hixon, and Lumen Lakes contained no records of fish stocking. The file for Zottle Lake could not be located. The year of stocking, number of fish stocked, and life stage stocked are given for each entry (f = fry, F = fingerling, A = adult).

	Acidic lakes				Circumneutral Lakes			
Species	**Hilderbrand**	**Currie**	**Flannery**	**Clara**	**Toulish**	**Dorothy Dunn**	**Nelson**	**Garth**
Cisco	--	--	--	--	--	--	1965-11,500f	--
Rainbow trout	--	--	1961-7700F 1962-7700F 1963-7700F	--	--	--	--	--
Northern pike	1968-174A 1975-200A 1975-200A 1976-200A	--	--	1954-18A 1956-172A	--	1937-194,000f	--	--
Muskellunge	--	--	1968-150F 1968-180F 1969-100F	--	--	1945-75F 1951-300F 1952-290F 1953-360F 1959-50F 1970-278F 1973-180F	1951-27F 1962-350F 1964-350F 1966-63F 1968-33F 1969-59F 1971-250F 1973-50F 1979-50F	1941-13,000f 1942-125F 1942-10,000f 1946-20,000f 1949-800F 1980-200F
Fathead minnow	--	--	--	1942-400A	--	--	--	--
Bluegill	--	--	--	--	--	--	1938-5000A 1939-400A 1939-1600F 1940-200A 1940-2300F 1941-1000A	--
Smallmouth bass	--	--	--	--	--	--	1942-125A 1972-500F	--

Appendix 16. Continued.

	Acidic Lakes				Circumneutral Lakes			
Species	Hilderbrand	Currie	Flannery	Clara	Toulish	Dorothy Dunn	Nelson	Garth
Largemouth bass	1944-75F 1945-500F	--	--	1942-80F 1947-1600F 1950-2600F 1952-430F	--	1939-??f 1941-1000f 1943-2000F 1944-150F 1945-2000F 1946-1600F 1949-1800F 1950-400F 1958-2400F 1968-2000F	1939-4000F 1940-117A 1941-2000f 1941-1000F 1941-1000F 1941-4500f 1942-23A 1943-3000F 1946-900F 1949-900F 1952-131F 1953-550F 1957-40F 1960-400F 1967-90F	1942-350F 1944-300F 1949-200F
Crappie[a]	--	--	--	--	--	--	1938-4000A	--
Yellow perch	--	--	--	--	--	--	1938-100,000F 1938-200A 1939-800F	--
Walleye	--	1980-4000f	--	1957-4700F	--	1975-7000F	1965-168F 1974-3000F	1941-500,000f 1942-160,000f 1948-500,000f
"Shiners"[b]	--	--	--	--	--	--	1941-16,000A 1941-1000F	--
"Chubs"[b]	--	--	--	1942-150A	--	--	1942-100A	--
"Suckers"[b]	--	--	--	1941-950A 1942-2000A	1941-117,000f 1942-25,000f	1944-375A	1941-2000F	--

[a] Genus *Pomoxis*; species unknown.

[b] The actual species and family of fish included in this grouping are unknown. Records for these entries should be interpreted with caution.

Appendix 17. Historical water chemistry data on six of the study lakes, collected by the Wisconsin Geological and Natural History Survey (E. A. Birge and Chancey Juday). A dash (--) indicates that no measurement was made. Data were provided by J. M. Eilers (Wisconsin Department of Natural Resources, Rhinelander).

Lake	Date	Secchi disc visibility (m)	Depth of sample (m)	Temp. (C)	pH	True color (Pt-Co units)	Specific conductance (µmhos/cm @ 20°C)	Dissolved oxygen (mg/l)	Carbon dioxide (mg/l)		Total P (mg/l)	Cl^- (mg/l)	$SO_4^=$ (mg/l)	NO_3^- (mg/l)	SiO_2 (mg/l)	Ca (mg/l)	Mg (mg/l)	Fe (mg/l)
									Free	Fixed								
Flannery[a]	17 July 1929	5.5	0	24.4	5.9	8	14	7.38	1.5	1.3	0.013	0.5	--	0.015	0.3	--	--	--
Dorothy Dunn	28 August 1926	2.3	0	21.0	7.3	14	32	8.62	2.20	7.37	0.020	0.9	--	0.000	2.0	--	--	--
			4	19.0	7.3	--	--	8.00	2.20	7.56	0.020	1.0	--	trace	2.0	--	--	--
			6	17.8	6.7	20	34	3.01	7.25	8.26	0.030	1.9	--	0.020	2.5	--	--	--
	27 June 1930	4.0	0	20.8	7.1	14	36	8.32	0.5	7.5	--	--	--	--	--	3.05	2.29	--
	15 August 1936	3.15	0	--	--	--	--	--	--	6.5	--	--	--	--	--	3.36	2.25	0.12
Garth	29 July 1928	2.2	0	21.2	7.3	14	72	6.97	1.3	15.5	0.015	1.0	2.27	0.012	5.0	8.80	8.03	--
Hixon	16 August 1936	--	0	--	6.7	43	35	--	--	7.25	--	--	--	--	2.6	4.44	2.25	0.52
Nelson	1 August 1930	4.0	0	23.0	7.1	10	37	7.6	1.0	9.4	--	--	--	--	6.0	4.9	--	--
Toulish[b]	23 August 1927	4.0	0	18.4	7.4	14	78	7.15	2.5	19.3	0.018	0.6	--	0.040	4.2	--	--	--

[a] Listed as Flanner in Birge and Juday records.

[b] Listed as Bolton Lake in Birge and Juday records.

Appendix 18. Common and scientific names of 27 species of fish collected in northern Wisconsin study lakes. Nomenclature follows Robins et al. (1980).

Family	Common name	Scientific name
Salmonidae	Cisco	*Coregonus artedii*
Umbridae	Central mudminnow	*Umbra limi*
Esocidae	Northern pike	*Esox lucius*
	Muskellunge	*Esox masquinongy*
Cyprinidae	Golden shiner	*Notemigonus crysoleucas*
	Blackchin shiner	*Notropis heterodon*
	Blacknose shiner	*Notropis heterolepis*
	Emerald shiner	*Notropis atherinoides*
	Weed shiner	*Notropis texanus*
	Bluntnose minnow	*Pimephales notatus*
	Creek chub	*Semotilus atromaculatus*
Catostomidae	White sucker	*Catostomus commersoni*
Ictaluridae	Black bullhead	*Ictalurus melas*
	Yellow bullhead	*Ictalurus natalis*
Gasterosteidae	Brook stickleback	*Culaea inconstans*
Centrarchidae	Rock bass	*Ambloplites rupestris*
	Green sunfish	*Lepomis cyanellus*
	Pumpkinseed	*Lepomis gibbosus*
	Bluegill	*Lepomis macrochirus*
	Smallmouth bass	*Micropterus dolomieui*
	Largemouth bass	*Micropterus salmoides*
	Black crappie	*Pomoxis nigromaculatus*
Percidae	Iowa darter	*Etheostoma exile*
	Johnny darter	*Etheostoma nigrum*
	Yellow perch	*Perca flavescens*
	Walleye	*Stizostedion vitreum vitreum*
Cottidae	Mottled sculpin	*Cottus bairdi*

Appendix 19. Numbers of fish collected during comprehensive (summer 1980) sampling of fish communities in 12 northern Wisconsin lakes by each gear type.

		Acidic lakes (pH shown in parentheses)						Circumneutral lakes (pH shown in parentheses)					
Species	**Gear[a]**	**Hilderbrand (5.1)**	**Lumen (5.2)**	**Currie (5.6)**	**Zottle (5.6)**	**Flannery (5.7)**	**Clara (6.0)**	**Toulish (6.7)**	**Hixon (6.8)**	**Clear (6.9)**	**Dorothy Dunn (6.9)**	**Nelson (7.0)**	**Garth (7.5)**
Cisco	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	26	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0
Central mudminnow	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	1	1	0
Northern pike	F	1	0	0	0	1	2	2	0	0	0	0	7
	G	10	0	0	0	0	18	16	1	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0
Muskellunge	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	7	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	4	0	0
Golden shiner	F	0	1	2	0	0	0	0	9	0	8	0	7
	G	0	1	0	0	0	0	0	9	0	1	0	1
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0
Blackchin shiner	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	20	0
Blacknose shiner	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	32	0	0	0	129	0

Appendix 19. Continued.

		Acidic lakes (pH shown in parentheses)						Circumneutral lakes (pH shown in parentheses)					
Species	Gear[a]	Hilderbrand (5.1)	Lumen (5.2)	Currie (5.6)	Zottle (5.6)	Flannery (5.7)	Clara (6.0)	Toulish (6.7)	Hixon (6.8)	Clear (6.9)	Dorothy Dunn (6.9)	Nelson (7.0)	Garth (7.5)
Emerald shiner	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	96	0	0
Weed shiner	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	6
Bluntnose minnow	F	0	0	0	0	0	0	2	0	3	0	0	4
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	39	14	165	0	26	1
	S	0	0	0	0	0	0	313	250	1017	0	1006	1073
Creek chub	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	1	0	0	0	0	0	0	0	0	0
White sucker	F	2	0	112	4	0	0	0	4	0	1	0	0
	G	4	1	103	11	2	0	2	0	43	15	12	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	2	0	0	0	0	0	1	1	0	0
Black bullhead	F	8	6	0	0	0	0	0	0	9	0	0	4
	G	0	1	0	0	0	0	0	0	0	0	0	1
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0
Yellow bullhead	F	0	0	0	0	110	171	3	91	18	2	1	68
	G	0	0	0	0	5	7	1	0	0	0	0	0
	M	0	0	0	0	1	0	0	0	1	0	0	2
	S	0	0	0	0	0	0	0	0	0	0	0	0
Brook stickleback	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	1	0

Appendix 19. Continued.

		Acidic lakes (pH shown in parentheses)						Circumneutral lakes (pH shown in parentheses)					
Species	Gear[a]	Hilderbrand (5.1)	Lumen (5.2)	Currie (5.6)	Zottle (5.6)	Flannery (5.7)	Clara (6.0)	Toulish (6.7)	Hixon (6.8)	Clear (6.9)	Dorothy Dunn (6.9)	Nelson (7.0)	Garth (7.5)
Rock bass	F	0	109	9	268	0	0	28	2	0	0	136	2
	G	0	9	0	20	0	0	9	0	0	0	4	0
	M	0	0	0	7	0	0	6	0	0	0	9	0
	S	0	0	0	8	0	0	2	0	0	0	4	0
Green sunfish	F	0	0	34	0	52	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0
Pumpinkseed	F	414	0	37	123	703	0	1	326	5	0	163	413
	G	13	0	0	2	46	0	2	6	0	0	0	13
	M	4	0	0	34	0	0	0	2	0	0	0	0
	S	93	0	5	34	37	0	17	54	0	0	25	48
Bluegill	F	39	8,481	172	0	420	569	452	655	122	16	576	1,671
	G	0	47	0	0	5	10	12	23	3	0	12	163
	M	0	2	0	0	12	109	46	35	1	0	69	7
	S	0	94	4	0	37	104	126	103	45	111	324	71
Smallmouth bass	F	0	31	0	0	0	0	4	0	0	0	2	1
	G	0	7	0	0	1	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0
Largemouth bass	F	1	115	3	0	6	14	9	1	2	3	5	4
	G	2	27	1	0	1	1	3	3	13	2	0	4
	M	1	0	9	0	0	0	2	5	1	0	3	9
	S	39	3	106	56	1	29	44	56	7	178	37	95
Black crappie	F	0	0	60	0	1	0	32	74	0	0	4	93
	G	0	0	8	0	0	0	2	10	0	0	0	10
	M	0	0	0	0	0	0	0	1	0	0	0	0
	S	0	0	0	0	0	0	1	0	0	0	0	0
Iowa darter	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	5	0

Appendix 19. Continued.

		Acidic lakes (pH shown in parentheses)						Circumneutral lakes (pH shown in parentheses)					
Species	Gear[a]	Hilderbrand (5.1)	Lumen (5.2)	Currie (5.6)	Zottle (5.6)	Flannery (5.7)	Clara (6.0)	Toulish (6.7)	Hixon (6.8)	Clear (6.9)	Dorothy Dunn (6.9)	Nelson (7.0)	Garth (7.5)
Johnny darter	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	1	9	6	0	0	0
Yellow perch	F	171	17	106	24	24	2	2	13	14	234	14	48
	G	779	280	623	721	520	277	27	118	154	286	73	899
	M	2	0	6	0	0	5	0	13	16	0	2	7
	S	17	2	2	2	0	1	9	15	26	161	15	76
Walleye	F	1	0	8	0	0	8	0	1	3	3	1	1
	G	0	0	4	0	1	15	0	0	6	11	7	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0
Mottled sculpin	F	0	0	0	0	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	1	0	0	0	0	0
	M	0	0	0	0	0	0	0	0	0	0	0	0
	S	0	0	0	0	0	0	0	0	0	0	0	0

[a] F = fyke net; G = gill net; M = minnow trap; S = shoreline seine.

Appendix 20. Total mercury concentrations in axial muscle tissue of individual walleye from northern Wisconsin lakes.

Lake	Fish identification number	Total length (mm)	Wet weight (g)	Age (years)	Mercury concentration (µg/g wet weight)
Currie (pH=5.6)	2	401	822.1	5	0.80
	1	484	1304.1	7	0.82
	5	426	735.8	5	1.41
	11	426	707.5	4	1.10
	6	442	877.3	7	1.11
	3	391	737.1	5	0.41
	4	384	481.1	4	0.72
	8	478	933.9	7	1.07
	9	500	1075.4	7	1.43
	12	372	481.1	4	0.50
Clara (pH=6.0)	20	466	1134.0	5	1.65
	7	401	652.1	4	0.58
	8	404	708.8	4	0.68
	3	399	822.2	4	0.49
	14	415	878.8	4	0.51
	10	426	765.4	4	0.57
	22	459	933.9	5	0.69
	16	531	1672.6	7	0.89
	21	540	1471.6	7	1.39
	15	458	1077.3	5	0.76
	13	467	1304.1	5	0.59
	17	521	1389.1	7	1.74
Clear (pH=6.9)	6	392	566.0	4	0.12
	3	401	566.0	5	0.24
	2	444	905.6	5	0.23
	5	375	537.7	4	0.17
	7	400	594.3	5	0.18
	1	469	933.9	5	0.21
	4	423	679.2	5	0.22
Dorothy Dunn (pH=6.9)	4	383	509.4	4	0.27
	6	366	396.2	4	0.22
	7	370	452.8	4	0.22
	11	477	1047.1	7	0.35
	12	391	566.0	5	0.31
	13	380	452.8	4	0.27
	14	419	622.6	7	0.32
	1	359	255.1	4	0.22
Nelson (pH=7.0)	3	426	707.5	7	0.57
	4	412	622.6	7	0.36
	2	320	371.3	5	0.38

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16. Abstract (Limit: 200 words)

Fish populations in six naturally acidic (autumnal pH 5.1-6.0) and six circumneutral (autumnal pH 6.7-7.5) clear-water lakes in north central Wisconsin were studied, and characteristics of fish populations and communities in the lakes were compared. The two groups of lakes were morphologically similar and of the same hydrologic types, but differed substantially in pH, alkalinity, waterborne calcium, and related chemical characteristics. Fish communities in the acidic lakes contained fewer species, and common as well as rare species occurred with lower frequency in the acidic lakes than in the circumneutral lakes. Certain taxa, such as minnows and darters were well represented in the circumneutral lakes, but were absent or scarce in the acidic lakes. Growth, condition, and serum calcium concentrations of bluegills were negatively correlated with population density and were not related to either pH or waterborne calcium concentrations in these lakes. Results of serum calcium analyses suggested that white suckers were stressed in lakes with autumnal pH equal to or less than 5.6 and waterborne calcium equal to or less than 2.0 mg/l. Mean concentrations of Cd and Pb in whole, 4-year-old bluegills were inversely correlated with both pH and waterborne calcium concentration. Mercury levels in axial muscle tissue of walleyes from two acidic lakes were substantially higher than in walleyes of the same age from three circumneutral lakes and exceeded the 1.0 ug/g USFDA Action Level in about 35% of the fish analyzed.

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